MOLECULAR BASES OF SCOUTING BEHAVIOR IN HONEY BEES

BY

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DISSERTATION

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ABSTRACT

Division of labor among honey bee foragers involves "scouts" and "recruits." Scouts seek new food sources or nest sites independently and recruit other bees in the hive to the locations of good food or nest sites instead of exploiting the resources themselves. In this dissertation, I hypothesize that scouting behavior in honey bees is analogous to novelty-seeking behavior in vertebrates, and is therefore associated with differences in brain dopaminergic, octopaminergic and glutamatergic systems. I found significant brain down-regulation of the D1-type dopamine receptor genes and upregulation of the octopamine receptor genes in scouts compared to non-scouts. Microarray analysis confirmed these findings and further implicated glutamatergic and GABAergic neurotransmitter systems. Oral pharmacological treatments using glutamate or octopamine both increased the probability of scouting, while dopamine antagonists decreased it. Blocking glutamate vesicle transport inhibited the behavioral effect of glutamate. I further hypothesize that scouts who seek food sources and those who seek nest sites would share a common "molecular signature" in their brains. Behavioral analysis showed that nest-site scouts were 3.4 times more likely to seek food sources later, and they shared a minimum of 89-gene expression profiles that predicted individual behavior with high accuracy. These findings illustrated how individual differences in behavior can arise from differences in gene regulation, and demonstrate intriguing similarities in human and insect novelty seeking, subserved by conserved molecular components. A shared molecular signature of scouting behaviors across ecological contexts also supports the scouting tendency as an "animal personality" and provides a molecular standpoint for studying the evolution of personality.

To my parents

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CHAPTER 1: GENERAL INTRODUCTION

Individual differences in behavior are prevalent among animal species, including our own. In humans, the combinations of unique emotional and behavioral responses of individuals are our personalities. In other animals, there are also individual behavioral differences that are consistent through changes in time and contexts [1]. We know that hereditary and environmental factors both influence these behavioral variations, but we understand little of their underlying molecular bases and neural mechanisms.

In honey bees, scouting behavior of adult worker bees is a critical component of both foraging and nest hunting [2, 3]. The tendency to scout for food or a nest site varies dramatically among individual bees. Scouting behavior is performed by scout bees, who fly out of the hive to search for novel resources independently, and recruit their nestmates to the most profitable sites via dance communication. Food scouts abandon the site they discovered while it is still profitable, to embark on new searches [2], which is contrary to the "win-stay, lose-shift" principle of foraging behavior [4].

Despite the differences in behavior and ecological contexts, both scouting behaviors share similar components with "novelty-seeking" behavior studied in humans and other vertebrates, a personality trait characterized by elevated exploratory activity in response to novel stimuli [5]. This dissertation was inspired by this similarity between scouting and novelty seeking, and it focused on using brain transcriptomic analyses and

pharmacological interventions to elucidate the molecular bases of scouting behaviors in the western honey bee, *Apis mellifera*.

1. Background

1.1 Individual Differences in Behavior

Many fields of research have made important contributions to the study of individual differences in behavior. Two fields of behavioral biology are of particular interests to my research: ecological research that studies correlated behavioral traits in individuals as behavioral syndromes or animal personality [1, 6], and behavioral genetics research that examines the genetic elements underlying human and animal behavior [7]. Each field has a distinct perspective and involves different selections of animal models and methodologies.

Animal personality research measures consistent and often correlative individual behavioral differences across time and contexts and seeks explanations for their adaptive values and evolutionary origin [6, 8]. Outbred animals in a natural population are studied in semi-natural environments, such as a tank or an animal's natural habitats. Classic examples are the studies of bold and shy personalities in three-spined stickleback fish [9, 10]. In general, animal personality research is less concerned with the molecular mechanisms underlying these differences. Behavioral genetics, on the other hand, focuses on elucidating genetic factors underlying complex behavioral traits, including traits related to individual differences in personality, intelligence, and mental disease susceptibility [7]. These studies mainly rely upon inbred animals studied under standard laboratory

conditions. Studies of the genetic basis of alcohol sensitivity in mice [11] and mutants affecting circadian rhythm in fruit flies are good examples of this type of research [12].

Molecular studies of individual differences in animal behavior in recent years have combined these two fields, using modern genetic and genomic analysis to study well-characterized natural behaviors in the wild. These efforts provide insights into both mechanistic and evolutionary aspects of behavior. Such examples include the genetic association of a variant of the dopamine receptor 4 gene (*drd4*) with exploratory behavior in juvenile great tits, *Parus major* [5], and the regulation of vasopressin receptor *var1* expression and pair bonding in voles [13, 14].

For animal societies such as honey bees, individual behavioral differences are mainly studied under the concept of division of labor [15]. My thesis initiated a new approach by considering the individuality of honey bees as being related to personality traits, such as novelty seeking in vertebrate species [16]. In addition, research by others has showed that honey bee colonies exhibit "collective personalities" – the colony-level behavioral differences in traits such as foraging activity and defense response. Differences in some of these collective behaviors were correlated with each other, consistent over time, and enhanced colony fitness [17]. Further study showed that swarming colonies consistently differ in their level of nest scouting activity, as well as in the number of waggle dances and shaking signals performed [18]. However, the "personalities" of individual honey bees or any mechanistic explanation for such personalities at either the group or individual level

were unknown.

1.2 Brain Transcriptomic Analysis

My studies of the molecular studies of scouting behavior took advantage of the sequenced genome of the western honey bee [19], and the increased capacity and analytical power to probe vast amounts of brain transcriptomic profiling data. I used brain gene expression profiling for the following reasons.

Changes in brain gene expression in specific brain regions, neural circuits, signaling pathways and across a time course, provide insights into molecular determinants of mental conditions and behaviors [20, 21]. For example, by using gene co-expression analyses, Voineagu et al. showed consistent differences in transcriptome organization between autistic and normal human brains and discovered discrete modules of co-expressed genes associated with autism in key brain regions [22]. In honey bees, using gene expression profiling of distinct behavioral phenotypes (foraging, aggression, and maturation), Chandrasekaran et al. constructed a brain transcriptional regulatory network (TRN) that showed specific neurogenomic states across distinct behavioral phenotypes [23].

Brain transcriptomic analysis also surveys the shifts of brain gene expression patterns in response to acute and chronic environmental stimuli, which mediate behavioral changes due to experience. Dong et al. reported thousands of gene expression changes in the auditory forebrain of zebra finches after they heard a new song versus an old song [24],

and Lutz et al. showed that similar changes occur in the mushroom bodies of the honey bee brain due to increasing foraging experience [25].

Brain transcriptomic study shows gene activities at the same time of behavioral response, which potentially signifies important spatial and temporal information of the behavior-related neural activities in the brain. Neurogenomic states associated with a behavioral variation may reflect both hereditary and environmental influences on the trait, and it links gene, brain and behavior in a way unreachable by traditional behavioral genetic approaches.

Despite the insights gained by using a transcriptomics-based approach, it is worth noting that this approach alone does not pinpoint the causative links between brain gene activity and behavior. Therefore, direct genetic and biochemical interventions, including mutations, RNAi and pharmacological treatments, are critical for identifying genomic elements, molecular pathways, or neural signing systems underlying behavior [26, 27]. In this dissertation, I focused on validating the causal link between brain gene expression patterns and scouting behavior by applications of pharmacological treatments.

1.3 Scouting Behavior

Scouting is a fascinating behavior in which a bee independently seeks out new resources without exploiting it, either nest-sites or food sources [2, 28, 29]. It is also an example of a behavior with inter-individual differences that leads to division of labor in the honey bee colony. Scout bees are typically foragers. While some of their unique behavioral features are studied, the molecular basis of scouting behavior was unknown. Scouting behavior is

an excellent model to study the molecular basis of individual differences in behavior for three reasons.

First, scout bees differ strongly from non-scout foragers in their motivation to seek novelty. Scouts independently seek new food sources or new nest sites, while most bees never do so; they leave the discovered site without continuing to exploit it, and in case of food scouts, continue searching for new food sources [28, 30, 31]. This individual difference is similar to novelty-seeking behavior in vertebrates, because scouts have an elevated exploratory tendency in response to novel stimuli and seem to prefer novelty to familiarity. Novelty seeking in humans is a personality trait and is correlated with genetic variations in some neurotransmitter systems. A human dopamine D4 receptor gene (*drd4*) polymorphism shows association with novelty-seeking behavior in humans [32, 33]. Both D1 and D2 types of dopamine receptors are involved in rodent novelty seeking [34, 35], and similar genetic associations with the dopamine system have been found in a variety of vertebrate species [36-38]. In addition, glutamatergic and GABAergic systems have also been implicated in human novelty seeking [39], and serotonin and acetylcholine have been reported to affect novel object discrimination or detection in rats, but it is not clear if the results were novelty-specific [40, 41]. These studies of novelty-seeking behavior across species offer us insights into the suite of candidate genes and pathways involved.

Second, scouting behavior is a natural behavior that is regulated by changes in social environment in addition to genetic predispositions. This makes scouting a good candidate for combining the research strategies of behavioral ecology and behavioral genetics.

During foraging, scouting activity is regulated by colony needs. As mentioned above, the proportion of foragers that scout can vary from 5% in summer, when floral sources are abundant, to 35% in late fall, when nectar availability sharply decreases [2, 28]. Scouting activity is also correlated with the number of active round or waggle dances performed by other bees in the hive, which serves as a mechanism to communicate about colony food needs [42]. These results suggest that the tendency of an individual to scout lies on a continuum, and there are not just two stereotypic extremes; scouts can emerge from a non-scout population if conditions warrant. On the other hand, opposite to Karl von Frisch's original belief that any bee can be a scout [43], distinct differences in scouting tendency can be observed in foragers at the first day of flight [44]. Both food scouts and nest scouts have been found to predominantly come from a subset of a colony's patrilines (honey bee colonies are typically genetically diverse with numerous patrilines because of queen polyandry) and have different genetic backgrounds from non-scouts [45-47]. These studies suggested that there are significant genetic factors associated with scouting behavior, suggesting a molecular basis for this trait.

Third, scouting behavior spans distinct ecological contexts, which makes it a good candidate for studying animal personality. This is because animal personality is defined as a consistent individual behavioral difference across time or contexts. Food scouts seek new flower patches and nest scouts search for unknown tree cavities and other potential dwelling places. It gives us the opportunity to test the correlation of two behaviors and, more interestingly, explore the molecular similarities associated with such correlations, which may reflect a shared novelty-seeking tendency across distinct behavioral contexts. If

two scouting behaviors have a shared molecular basis, an individual food scout should also behave as a nest scout, and *vice versa*. Observations suggest that foraging stops when nest hunting begins [48], providing a suggestive link between these two forms of scouting. Because scouting behavior is a critical part of the honey bee social foraging system [2], scouting behavior is clearly adaptive. Hence, molecular studies of scouting behavior can contribute to the important study of the mechanism underlying correlated behavioral traits, their adaptive value, and the evolution of animal personality in social species [49, 50].

2. Hypotheses and Chapters

Following this chapter are two chapters that describe my experimental work. Together, they tested three hypotheses about scouting behavior in honey bees:

1. Novelty-seeking hypothesis:

Scouting behavior is analogous to vertebrate novelty-seeking behavior.

2. Molecular hypothesis:

There are molecular determinants that mediate novelty seeking in the scout brains.

3. Animal-personality hypothesis:

Scouting is a consistent individual difference across distinct behavioral contexts.

Chapter 2 has been published in 2012 [51]. It tested the first and second hypotheses, as well as the behavioral aspect of the third hypothesis. Chapter 3 is in preparation for submission and focuses on testing the molecular aspects of the third hypothesis, which supports and extends the findings from studying the first two hypotheses.

Chapter 2 reports a series of studies in search of the molecular determinants of scouting behavior in honey bees. In this chapter, I first tested if there was consistency in the scouting tendency of individual bees across foraging and nest hunting contexts. The results showed that nest scouts were far more likely to become food scouts compared to control swarming bees, suggesting that scouting is a personality-like trait. Second, based on the hypothesis that scouting behavior in honey bees is analogous to novelty-seeking behavior in vertebrates, I developed a new behavioral assay to test novelty seeking more directly than previous scout assays in semi-natural conditions. After successfully identifying reliable scouts and non-scouts using this assay, I performed a whole genome microarray analysis to compare scout brain gene expression patterns to those of non-scouts. As a part of large neurogenomic signature for scouting behavior in the bee brain, I surveyed 10 neurotransmitter-related genes in the glutamate, dopamine and octopamine neurotransmitter systems and found that their brain gene expression patterns were sufficiently different to classify individual bees as scouts or non-scouts. Encouragingly, the same was true for scouts and non-scouts identified using a second scout behavioral assay. I used this second method in pharmacological experiments to test scouting probability after treatments.

In order to support a causal link between neural signaling genes and scouting behavior, I used a variety of neurochemicals to manipulate scouting probability in non-scouting bees, targeting four neurotransmitter systems in the brain: dopamine, glutamate, octopamine and GABA systems. The treatment protocol combined laboratory and hive conditions to maximize drug consumption and survival rate. The behavioral assay reliably tested

changes in scouting probability for a large number of bees in the field. By analyzing results over two years, I found that glutamate specifically increased the scouting tendency in non-scouting foragers, and I identified a subtle increase caused by octopamine. Both influenced scouting probability in a dose-dependent manner. I also found that dopamine antagonists decreased scouting probability of non-scouting foragers. Taken together, these results support the idea that individual differences in scouting behavior reflect an animal personality trait and are influenced by monoamines and glutamate, also among the neurotransmitters that influence human novelty seeking.

Chapter 3 presents results of comparative brain transcriptomic analyses of nest and food scouts in their respective contexts. If scouting in honey bees is a behavioral syndrome or personality trait, then we should observe common patterns of brain gene expression for both types of scouts, despite the differences in behavior and context. Because of the differences in behavior and context we would not expect identical patterns of brain gene expression. Instead, we would expect a common core of genes showing similar patterns across both types of scouting, which reflects the shared novelty seeking in different types of scouting behaviors. In this study, I tested this hypothesis using a whole genome microarray for an unbiased survey of brain gene expression. I compared scouts and recruits in both contexts: food scouts and food recruits during foraging and nest scouts and nest recruits during nest hunting.

This chapter has three principal findings. First, I found that there are indeed shared brain expression patterns for scouting behavior across contexts. This result supports the idea that

scouting in honey bees is a behavioral syndrome or animal personality trait. Second, I confirmed that genes involved in monoamine, aceytylcholine, glutamate and GABA signaling, the same pathways that influence novelty seeking in food scouts, were also shown in this study to clearly distinguish scouts and recruits in both scouting contexts. Evidence for differences in cholinergic –related gene expression was also observed in an update of the results in Chapter 2 using a more recent Official Gene Set (3.2) of the honey bee genome than what was available at the time Chapter 2 was published. Third, I identified a molecular signature of a minimum of 89 genes that was sufficient to predict a bee's role as a scout or a recruit with 92.5% success. These findings of the shared molecular signature of scouting behaviors across different ecological contexts support the idea that scouting tendency is an animal personality. Moreover, as I discuss in Chapter 3, shared molecular mechanisms underlying correlated behaviors may provide some explanations for the limited plasticity of personality.

3. Conclusion

This dissertation implements a broad range of approaches from neuroscience, animal behavior and genomics to elucidate the molecular basis of a fascinating natural behavior. This dissertation makes original contributions to the field of neurobiology and animal behavior by demonstrating the molecular basis of an animal personality, showing that it is mediated by a convergent brain transcriptomic signature in distinct ecological contexts, showing an intriguing molecular conservation underlying both insect and human personalities, and providing molecular insights to our understanding of the adaptive value of personality in social life.

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CHAPTER 2: MOLECULAR DETERMINANTS OF SCOUTING BEHAVIOR IN HONEY BEES

Previously published work[1]

ABSTRACT

Little is known about the molecular basis of differences in behavior among individuals. Here we report consistent novelty-seeking behavior across different contexts among honey bees in tendency to scout for food sources and nest sites, and we reveal some of the molecular underpinnings of this behavior relative to bees that do not scout. Food scouts showed extensive differences in brain gene expression relative to other foragers, including differences related to catecholamine, glutamate, and GABA signaling. Octopamine and glutamate treatments increased the likelihood of scouting, whereas dopamine antagonist treatment decreased it. These findings demonstrate intriguing similarities in human and insect novelty seeking and suggest that this trait, which presumably evolved independently in these two lineages, may be subserved by conserved molecular components.

[1] Liang ZS, Nguyen T, Mattila HR, Rodriguez-Zas SL, Seeley TD, Robinson GE, *Science.* 335:1225-1228 (2012). ZSL and GER conceived the project, designed the experiments and wrote the paper; ZSL performed sample collection, molecular and field experiments and analyses; TN and SRZ performed microarray experiments and statistical analyses, respectively; HRM and TDS contributed to protocol development, sample collection and co-wrote the paper. Reprinted with permission from AAAS: http://www.sciencemag.org/site/feature/contribinfo/prep/lic_info.pdf

INTRODUCTION

An important challenge in behavioral biology is to elucidate the molecular basis of individual differences in behavior. Scouting behavior in the honey bee, *Apis mellifera,* provides an excellent opportunity to explore this issue for two reasons. First, there are striking individual differences in this behavior—some bees act as scouts while others never do so. Second, scouting is performed in two distinct contexts, for new food sources or new nest sites, which suggests an underlying tendency to seek something new. Novelty-seeking behavior has been studied in vertebrates, including humans (*1,2*), but not in insects.

Food scouts, comprising 5-25% of a colony's foraging force, search independently for new food sources, and continue to do so even when plentiful sources have been found *(3-5)*. Non-scouts do not search for novel food sources and instead rely on information from scouts (communicated via "dance language") to guide their foraging. By constantly discovering new flower patches, food scouts help ensure a high influx of food to their colony despite the ephemeral nature of each patch *(5)*.

Nest scouts comprise <5% of the population of a swarm, which is a fragment of a colony that has left its natal nest to start a new colony. Nest scouts search independently for potential nesting cavities and collectively choose the best one while non-scout swarm members rely on information from scouts to guide them to their new home (*6*). Nest scouting also is a crucial behavior; a colony's survival depends upon its nest scouts finding suitably protective living quarters.

RESULTS

To determine the consistency of novelty seeking in individual bees across the two behavioral contexts, we determined whether nest scouts are prone to also act as food scouts. We identified and marked nest scouts in both artificial and natural swarms (*6*). We then identified food scouts with the standard "hive-moving" assay *(5,7*), after installing each swarm in a beehive and moving it at night (when bees don't forage) to a new location outside the bees' original home range. This assay identifies food scouts as the first bees to return to their hive in the morning; under these circumstances each successful forager must have located a food source on her own. There was a robust tendency of nest scouts to seek novel resources across different contexts, but it did not translate into every nest scout showing food-scouting behavior. In 9 trials involving 8 different colonies over two years, nest scouts were on average 3.4 times more likely to become food scouts than were bees that did not search for nest sites during swarming (Fig 2.1A). These results demonstrate that some bees show consistent novelty seeking across diverse behavioral contexts.

To explore the molecular basis of novelty seeking in bees, we developed a behavioral assay for food scouts (Fig 2.1B) that tests novelty seeking more strongly than previous scout assays *(3,5,7)*. A large screened outdoor enclosure provided experimental control of food sources under otherwise naturalistic conditions. Foragers from a glass-walled observation hive were trained to a "training feeder" that initially was the only food source available to them. After 2-3 days of training, a "novel feeder" with different visual and odor cues was placed at another location in the enclosure. The foraging bees thus had two possible food sources, familiar and novel; some bees discovered the novel feeder and

switched to it. This procedure was repeated on several consecutive days, and each time the novel feeder was given new visual and odor cues and placed in a new location. Only bees that switched to two or more different novel feeders, after being seen at least once at the training feeder, were collected as scouts. These rigorous criteria minimized the possibility of identifying scouts on the basis of an accidental discovery of a novel feeder, given the relatively short distances between feeders. The proportion of scout bees identified with this assay (31.2%, ± 9.7% SD, n = 182 bees, 6 trials) is roughly consistent with what has been observed under more natural conditions *(3-5),* suggesting that accidental discoveries of novel feeders was not a major source of error. Bees that met our criteria for identifying food scouts were collected to compare brain gene expression with control "non-scouts" (foragers that never were observed to switch to a novel feeder).

Whole genome microarray analysis revealed a large neurogenomic signature for scouting behavior in the bee brain. Sixteen percent (1219 out of 7539) of the transcripts on the microarray showed significant (False Discovery Rate, FDR < 0.05) differences in mRNA abundance between scouts and non-scouts (Tables 2.3, 2.4, 2.5). Among the differentially expressed genes were several related to catecholamine, glutamate, and GABA signaling, which are involved in regulating novelty seeking and reward in vertebrates (*1,2,8*). For example, the down-regulation of a dopamine receptor gene in honey bee scouts parallels results for a similar gene in individual mammals that are prone to novelty seeking *(9)*. These signaling systems also are implicated in personality differences between humans related to novelty seeking (*10,11*).

Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) analysis confirmed the microrarray results for five genes related to catecholamine, glutamate, and GABA signaling (Fig 2.1A, Figs 2.4 A and B): D1-type dopamine receptor *DopR1,* glutamate transporters *Eaat2* and *Vglut*, AMPA-type glutamate receptor *Glu-RI*, and GABA transporter *Gat-a*. Three additional catecholamine receptor genes also were differentially expressed but undetected in microarray analysis: *DopR2* (D1-type) *(12)*, *Octß2R* (ß-adrenergic type octopamine receptor), and *OctR1* (α-adrenergic type) *(13)* (Fig 2.4B, Tables 2.1, 2.2).

Linear Discriminant Analysis (LDA) showed a strong separation between scouts and non-scouts based on the expression values for 10 neural signaling genes related catecholamine, glutamate, and GABA signaling (Fig 2.2B). These 10 genes also showed strong similarities in brain gene expression for scouts identified either with the new feeder-discovery or the hive-moving assay (Fig 2.5).

The association between scouting and catecholamine, glutamate, and GABA signaling pathways could reflect effects of this behavior on brain gene expression, or effects of individual differences in these pathways on scouting, or both. We used the transcriptomic results as the basis for designing experiments to test causal relationships, hypothesizing that neurochemical treatment would influence scouting behavior. We tested this hypothesis with the hive-moving assay because it results in rapid identification of numerous scouts. We collected non-scouts and provided them with a chronic oral treatment (25-30 h) in cages (20 bees/cage) in their hive before moving it overnight to a location outside the

colony's original home range.

Behavioral observations the following morning (14 h after stopping the treatment) revealed that glutamate (monosodium glutamate, MSG) caused a significant increase in scouting (Fig 2.3A), while vesicular glutamate transport blocker Chicago Sky Blue significantly attenuated the MSG effects (Fig 2.3B). Octopamine caused a weaker, but still significant, increase in scouting (Fig 2.3A). These results are consistent with predictions based on microarray analysis. By contrast, dopamine antagonists caused a significant decrease in scouting (Fig 2.3C), which was contrary to microarray-based prediction. Effects were not seen in all trials (Figs 2.6, 2.7, 2.8), suggesting factors such as food availability, colony conditions, worker genotype, or other unknown variables also affect the probability of becoming a scout. The treatments did not cause excess mortality (Table 2.7), aberrant locomotion, hyperactivity, or a general increase in foraging activity (Fig 2.9), and they were dose dependent (Fig 2.10), which suggests that there were specific treatment effects on scouting behavior. GABA or GABA receptor agonist (TACA) did not affect the probability of scouting (Fig 2.11), so the role of this neurotransmitter in bee scouting remains unclear.

Multiple neurotransmitter systems appear to be involved in the regulation of scouting in honey bees, but it is not known how they interact at the circuit level. Glutamatergic and dopaminergic neurons are both found in the vertical lobes of the mushroom bodies, a part of the insect brain involved in reward learning *(14,15)*. *DopR1* and *Eaat-2* gene expression is co-localized to the same type of interneurons that provide sensory input into these lobes

(16,17). These findings, together with our own, suggest the vertical lobes of the mushroom bodies as one possible neuroanatomical locus for novelty-seeking behavior in honey bees, though other brain regions are likely involved as well.

DISCUSSION

Our results demonstrate intriguing parallels between honey bees and humans in novelty-seeking behavior. Though the molecular machinery that produces this behavioral variation is similar, but it is unknown whether both species inherited them from a common ancestor or whether they evolved them independently. Given the phylogenetic separation of bees and humans, we believe it is likely that these mechanisms represent part of a basic "toolkit" that has been used repeatedly in the evolution of behavior. Further support for this view comes from the finding that individual differences in food-searching behavior in nematodes (*Caenorhabditis elegans)* are caused, in part, by non-coding polymorphisms in *tyramine receptor 3*, which encodes a receptor for a catecholamine closely related to octopamine and dopamine (*18*).

It is common to look to animal models to generate insights that may be applicable to human behavior. Our findings highlight the potential of the converse—using insights from human research to further elucidate the molecular basis of animal behavior. Animal studies, informed by inferences from human research, might in turn help identify evolutionarily conserved molecular mechanisms underlying consistent differences among humans in various behaviors, thus helping us better understand how and why these behavioral differences exist.

MATERIAL AND METHODS

Bees

Bees were maintained at the University of Illinois Beekeeping Facility and the Cornell University Liddell Field Station according to standard beekeeping practices. For the novelty-seeking assay, six colonies were used at Illinois, each headed by a queen that was instrumentally inseminated with semen from a single, unrelated, male (SDI colonies), to minimize effects of genetic variation within each trial. Because of haplodiploidy the average coefficient of relatedness among workers within each colony was 0.75. Collections were limited to bees foraging on artificial nectar (sugar syrup), and only bees collected in the morning were used, to control for diet and circadian effects, respectively. For the pharmacological studies using the hive-moving assay, a total of 21 colonies were used at Illinois, each headed by a naturally mated queen. Bees used for molecular validation of the hive-moving method (which involved comparing brain gene expression between scouts collected in the feeder-discovery and hive-moving assays) were collected from three unrelated SDI colonies at Cornell.

Scouting Assays

1. Feeder-discovery Assay. A 4-frame glass-walled observation hive (containing ca. 8000 bees) was placed at the center of a large outdoor flight cage (6 m wide x 20 m long x 3 m high). Previous studies have shown that honey bees exhibit typical foraging behavior in this type of enclosure *(S1, S2).* Initially, foragers were trained to an artificial sugar-water feeder from 9.00-15.00 for two or three days. The training feeder provided unscented 50% sugar syrup (v/v) and a yellow-colored floral pattern. All foragers visiting the training

feeder were marked with a spot of paint on the dorsal surface of the thorax. On Day 1 of the experiment, the training feeder was set up as before but 30 min later a novel feeder was presented, on the side of the flight cage opposite to the location of the training feeder (Fig 2.1A). The novel feeder provided the same concentration of sugar syrup, but was slightly scented with an odor (e.g., orange scent) and was marked with a different visual pattern (e.g., a blue-yellow checkerboard pattern). All foragers that discovered the novel feeder on Day 1 were paint-marked, collected, and held in plastic cages to prevent them from recruiting other foragers to the novel feeder via the waggle dance. The cages were kept in a 34°C incubator during the experiment (1-6 h); the bees were released to their colony at the end of the experimental period (15.00-17.00). The training feeder was open during the entire experimental period (9.00-15.00) and all foragers that visited this site were marked as before. On Day 2 we followed the same procedure as Day 1, but a new novel feeder was set up (the old one having been taken down at the end of Day 1). This novel feeder was in new location in the flight enclosure, with a new scent and new visual pattern and color. Again, all foragers that discovered the novel feeder on Day 2 were paint-marked with a different color to that used on Day 1. “Scouts” were defined as foragers that found both novel feeders independently on successive days after also being seen at the training feeder. In some trials, this procedure was extended a few more days (and with more novel feeders) to ensure adequate sample sizes for molecular analysis. “Non-scouts” were defined as foragers that only visited the training feeder and were never seen at any of the novel feeders. They were collected on the last day of each trial. Only the morning collections of each behavioral group were used for molecular analysis.

2. Hive-moving Assay. The hive-moving assay was used mainly to assess the effects of pharmacological treatments on the probability of expressing scouting behavior. But before using the hive-moving assay to identify food scouts in the pharmacology experiments, we compared the scouts obtained with this assay to those of the novelty-seeking assay. We did so by measuring brain gene expression in scouts collected in both assays. This was done because our choice of pharmacological treatments was based on the results of gene expression analyses that were done on scouts collected with the novelty-seeking assay. Each colony used for molecular validation of the hive-moving assay had its hive entrance screened and then was moved to a new location in the evening, at least 4 km away from the colony's original location in order to get beyond the home range of the foragers and thus present them with an entirely new foraging environment *(S3)*. The following morning hive entrances were opened at 8.00 and scouts were collected for 1 h. Scouts were identified as the first bees to leave the hive, forage in the unfamiliar environment and return to their hive with food *(S4, S5)*. To prevent recruitment, we blocked the hive entrance so that foragers could leave but not return (*S6*). Analysis of each returning bee's foregut contents was performed to verify scout status (*S5)*.

For pharmacological experiments, the hive-moving assay was performed as above except for a difference in the duration of the experiment and the distance of the move. More details, including scouting probability calculation method, are given in "*Pharmacological Experiments"* section.

Nest Scout-to-Food Scout Experiments

1. Natural Swarms. We used four naturally occurring swarms that emerged in May and June from two colonies in beehives (three swarms) and from one colony in a tree (one swarm). We arrived at each swarm's clustering site before the active dancing phase had started, and we were able to find the queen and cage her to prevent the swarm from leaving during the experiment. The experiment was conducted either directly at the site where the swarm landed, or after we transferred the swarm to a "swarm mount" (*S7*), i.e., a wooden board mounted vertically on a 1-m tall stick. Either way, we began by waiting for scouting activity to occur. Scouts were identified as the bees performing waggle dances during the initial searching phase, when the bees' dances indicated sites in various directions. Each scout was marked with a spot of paint on her thorax and was allowed to continue her activity. We marked all the scouts that we could find on the swarms. Control bees were swarm bees that were roughly the same age as the scouts (i.e., older bees, as indicated by noticeable wear on their wings) but were not acting as scouts, and instead were motionless or walking slowly on the surface of the swarm cluster. Control bees (about the same number as scouts) also were paint marked. After both groups were identified, the swarm was carefully shaken into a standard hive box with five frames of combs (one or two with food - honey and pollen, the rest empty) and the queen was released into the colony. The hive was sealed after dark (when all bees were inside) and moved to an unfamiliar location at least 6 km away. Food scouting by the marked bees (scouts and control bees) was then assessed in the morning at the new location using the method described above. Food scouting was assessed on the first morning after the swarm was hived in Trials 2 and 3, but for Trials 1 and 4 the food scouting was assessed 2 days and 3 days, respectively, after

hiving the swarms, because of poor weather. The probability of food scouting by the bees in each group was calculated as the proportion of the marked bees in a group (scouts or controls) that were identified as food scouts.

2. Artificial Swarms. Artificial swarms were prepared according to standard procedures (*S8*), during the natural swarming season of June and July. A different colony with a naturally mated queen was used in each artificial swarm trial. For each colony, we first located it's queen and put her in a small cage (3.2×10×1.6 cm). Then, using a large funnel, we shook 1.5 kg of bees (~12,000) into a wooden "swarm box" (15×25×35 cm) with screen wire sides, and placed the caged queen inside it. To obtain both young bees and older bees (foragers), we shook bees off frames located in both the upper and lower parts of the hive in the late afternoon-early evening, when foraging had largely ceased for the day. The swarm box was then placed in a dark room at 18-23°C for 3-5 days, with *ad libitum* sugar syrup (50% m/v) available through a gravity feeder fit on top of the box. Twice a day, the screen sides were also brushed with the same syrup. The swarm box was kept in the dark room until numerous wax scales had dropped from the bees. The artificial swarm was then transferred to a swarm mount outside. The queen cage was hung in the upper center of the board, and a feeder was placed on top of the board as a temporary food source for the swarm. We then carefully opened the swarm box and shook all the bees at the base of the swarm mount stand. Within about 1 h the bees were clustered around the queen as in a natural swarm; nest scouting activity usually started 0.5-1 h after the cluster was formed. Behavioral identification of nest scouts and control bees, and assessment of food scouting probability, were performed as described above.

Analysis of Gene Expression

1. Sample Collection and Brain Dissection. Bees collected in the field were immediately dropped into liquid nitrogen to preserve natural levels of brain gene expression. Heads were freeze-dried at -80°C and then whole brains were dissected out in 100% ethanol and stored at -80°C *(S9)*.

2. Quantitative RT-PCR. RNA was isolated from whole brains using TRIzol® Reagent (Invitrogen, CA) and RNeasy® Mini Kit (Qiagen Sciences, Maryland). RNA extraction was carried out as per manufacturer's instructions for total RNA and for on-column DNase I treatment (Qiagen). RNA yields and purity were determined with a NanoDrop® ND-1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Delaware). cDNA was synthesized from 200 ng RNA. Primers for each gene were designed with Primer Express software (Table 2.1). Real-time qPCR was performed with an ABI Prism HT7900 sequence detection system with SYBR green detection (Roche Diagnostics, Indiana). An exogenous control gene, *Arabidopsis root cap protein 1* (*rcp1*), was spiked into each sample during the RT reaction to control for differences in cDNA synthesis. Expression values were normalized to the expression of an internal control gene (*eif-S8)* or exogenous control gene *rcp1* on an individual sample basis.

A two-way ANOVA was used to analyze the qPCR gene expression data. The model included the fixed effects of behavior group and colony and their interaction (*p*-values reported in Table 2.2). Outlier observations were removed based on extreme studentized residuals. Analysis and post hoc adjustment were implemented using SAS 9.1, PROC

GLM. Standardization of microarray and qRT-PCR gene expression data (Fig 2.5) was performed in SAS 9.1 using PROC STANDARD.

3. Microarray Procedures. We used the honey bee "whole genome" microarray, which was designed primarily based on Honey Bee Genome Sequencing Consortium "Official Gene Set" *(S10)* and previously characterized *(S11)*. Forty bees were used, 20 food scouts and 20 non-scouts, sampled from three SDI colonies (Year 2 samples in Fig 2.4). They were compared in an interconnected loop design with a total of 58 microarrays. 500 ng RNA per bee brain were amplified using MessageAmpII (Ambion/Applied Biosystems, Austin, Texas) kits. Samples of 2.5 ug amplified RNA were used for microarray hybridization. Dye coupling and labeled aRNA cleanup were carried out with the Kreatech ULS labeling system (Amsterdam, The Netherlands). Labeled aRNAs were hybridized onto microarray slides following standard protocol *(S11)*. Hybridized arrays were scanned by Axon 4000B scanner and the images analyzed using GENEPIX software (Agilent, California).

4. Microarray Analysis. Fluorescence intensity spots were filtered if flagged by the scanning software or if the intensity did not surpass the median intensity of the negative control spots for each channel. A Loess transformation was used to adjust the log2 transformed gene expression intensities for dye effects within microarray, and duplicated spots within microarray element were averaged. Gene intensities within each microarray were centered to allow analysis across microarrays, and microarray elements with more than one missing observation were removed from the analysis.

Statistical analysis of the microarray data was performed with a linear mixed effects model implemented using REML to describe the normalized log2 transformed gene intensities values, including the effects of dye, behavioral group, bee and microarray. F1-type False Discovery Rate *p*-values including multiple-test adjustment *(S10)* was used to generate lists of differentially expressed genes.

A total of 12661 probes remained after these processes; 7539 probes represented genes that were expressed on ≥ 56 microarrays (out of 58 total, 97%); others were excluded from further analysis. Genes that were known to be highly expressed in the hypopharyngeal glands were also excluded due to the risk of contamination during brain dissection *(S12)*. Differences in mRNA abundance were evaluated with an F-test statistic; False Discovery Rate p-values include multiple-test adjustment *(S11)*. Gene Ontology analysis was performed with DAVID bioinformatics resources 6.7 (http://david.abcc.ncifcrf.gov) (*S13*) and BiNGO 2.3 plugin in Cytoscape software (version 2.6.1) *(S14)*. Linear discriminant analyses were performed using the "lda" function in MASS package of R software (version 2.10.1). Hierarchal clustering and heatmap were generated in GENESIS software *(S15)*.

Pharmacological Experiments

1. Forager Collections. Foragers returning to the hive were collected from 11.00-15.00 at the hive entrance. Care was taken to avoid the time of day when most younger bees take "orientation flights" to avoid collecting them in the forager samples. We focused our treatment experiments almost exclusively on nectar foragers that were non-scouts.

Foragers with pollen loads were excluded, and a random sample (n = 20) of the remaining bees revealed large nectar loads in their foreguts (*S16*).

2. Oral Treatments. Bees were chilled in groups of 30-50 at 0° C for ca. 15-20 min immediately after collection to facilitate paint marking and caging. Bees were randomly assigned to a treatment group and paint-marked accordingly. Groups of 20 foragers were placed together in a Plexiglas cage (10 × 10 × 7 cm) and compounds were administered orally through two feeder tubes modified from 1.5 ml microcentrifuge tubes. Caged bees were then kept temporarily in a 34ºC incubator to allow for recovery from chilling, and the time they became mobile was noted as the start time of the treatment. Compounds were dissolved in 50% sugar syrup (m/v), which was administered *ad libitum*. A total of 10-12 cages, i.e., 200-240 bees, were used for each treatment group in each trial of the experiment. Each trial used bees from a different colony and contained 2-3 treatment groups and one control group, thus there were 30-48 cages per colony, filled with 600-960 bees for each experiment. After all the cages were filled with bees (incubation time ranged from 10 min to 4 h), the treatment cages were then placed back in the hive from which the bees originated. To accommodate the treatment cages, one empty hive box ("treatment box") was added on top of each beehive with a screen on the bottom to separate it from the colony; and the treatment cages were placed inside it. In this way, all the focal bees were maintained inside their own hive.

3. Post-treatment Handling. Treatments ended shortly before sunset on the following day (18.00-19.30), giving the bees a 25-30 h chronic treatment. At this point, the treated bees

were released back to their colony in two steps. First, we opened the treatment box and then opened the cages on top of the box to allow the bees to exit on their own, then we shook and brushed all the bees onto the screen that divided the treatment box and the colony, carefully removed the screen, and transferred the bees back into their colony. Before releasing the bees, we checked all the cages for syrup (control and drug) consumption, survival numbers (Table 2.7) and general activity of the caged bees. Slight, mostly non-significant, differences in consumption were noted (Table 2.7); survival rates were equivalent (Table 2.7). Bees that either could not move back to their hive or were rejected by their colony (less than 2%) were counted and subtracted from total survival numbers.

4. Hive Moving and Measuring Probability of Food Scouting. After the treated bees were released from the cages and given adequate time (0.5-1 h) to move back to their hive, the hive was sealed and moved at night to a site 6-12 km away from the colony's original location. Scouting probability was then measured the following morning starting between 8.00-9.00, hence about 14 h after treatment. Bees returning in the first 15 min were excluded to ensure that we were sampling foragers; shorter flights may represent re-orienting bees. After that, all returning bees were collected and their returning times and paint marks were recorded. This protocol continued for 2.5-3 h. Scouting probability was calculated as the proportion of foragers in each treatment group that exhibited scouting behavior:

Scouting probability $P = N_1$ (number of bees that scouted) / N_2 (number of total bees

available)

Casual observations revealed that the bees that we identified as scouts tended to have small nectar and pollen loads; this is consistent with previous observations of scouting behavior *(S5)*. These observations, plus the fact that the hive entrance was blocked to prevent returning foragers from entering the hive (where they might recruit others) suggests that our sampling of scouting behavior was accurate. The same procedure was also used in the nest scout-to-food scout experiments to assess the food scouting probability of nest scouts.

6. Tests for Increase in Non-scouting Foraging. To determine the effects of the above treatments on general foraging activity, we conducted a second trial of the above experiment with each colony, but in these second trials we did not block the hive entrance after we moved the hive and thereby allowed returning scouts to freely recruit nestmates the following morning. Treatments did not cause an overall increase in foraging activity relative to control bees (Fig 2.9), which suggests that the effects of MSG and OA on scouting probability were not due to a general increase in foraging activity.

Sampling during 13 trials (n = 573 bees) revealed that about half of the bees that we identified as scouts returned to their colonies with loads of nectar after being relocated to a novel foraging environment, validating their status as scouts (Table 2.6). The remaining bees probably were unsuccessful scouts; since they were all identified as foragers prior to pharmacological treatment, it is unlikely that they left their colonies for reasons other than to forage *(S17)*.

6. Statistical Analyses. A mixed-effect model ANOVA including treatment (MSG or OA) as fixed effect, colony and year as random effects, was used to analyze the probability of scouting following treatment. This model allowed testing the overall effect of treatment while controlling (blocking) for colony and year. Data were square root transformed to best fit the assumptions of the model. Analysis and a post-hoc adjustment (Tukey-Kramer) were implemented using SAS 9.1, PROC MIXED. A similar model was implemented for another treatment (MSG vs. MSG+CSB) as a fixed effect, colony as a random effect, using untransformed data. To test the effect of dopamine antagonists, a mixed-model ANOVA was used, including the fixed effects of treatment and random effect of colony, with *post hoc* Tukey adjustment for multiple testing. Because fewer colonies were tested, we used the square-root of the covariance estimation for residual / number of trials per group to estimate the error term. Fisher's Exact Test (GraphPad.com, GraphPad Software, Inc, California) was used to test the significance of the treatment effect for each individual trial. Two-tailed *p*-values were reported unless indicated otherwise.

Additional Microarray Results

Gene Ontology functional analysis was performed using 670 genes with high-confidence orthologs in *Drosophila melanogaster* (circa May 2009), out of 1219 differentially regulated transcripts (FDR<0.05, Table 2.4 as Appendix A). Using as a basis 4173 fly orthologs (out of 6419 genes that were expressed consistently on the microarrays; signals were detectable for 97% of the arrays in the experiment), overrepresented GO terms in lists of up- and down-regulated genes were identified, with redundant terms removed by GO

FAT in DAVID version 6.7. Enrichment analysis was performed in three ways: 1) DAVID version 6.7 with EASE score (Fisher's Exact Test, *p*-value <0.01) generated the terms shown in Table 2.5. 2) The same test with Benjamini correction $p<0.05$ generated a more stringent list, highlighted in yellow in Table 2.5. 3) Hypergeometric test with FDR <0.05 by BiNGO generated a nearly identical list of enriched terms (not shown) as did #2. Six KEGG pathways were identified from the enriched GO terms (Table 2.5).

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FIGURES AND TABLES

Figure 2.1 (A) Consistent novelty-seeking behavior across different contexts. Nest scouts were significantly more likely to later act as food scouts than were control swarm bees (non-scouts on swarms). Graph shows the probabilities of food scouting for 9 trials: 4 natural swarms and 5 artificial swarms, with 8 different colonies (Fisher's exact test, 2-tail, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$), and the overall mean probabilities (least-square means and standard errors, mixed-model ANOVA, 2-tail). **(B)** Feeder-discovery assay for identifying food scouts. See text for description; additional details in SOM.

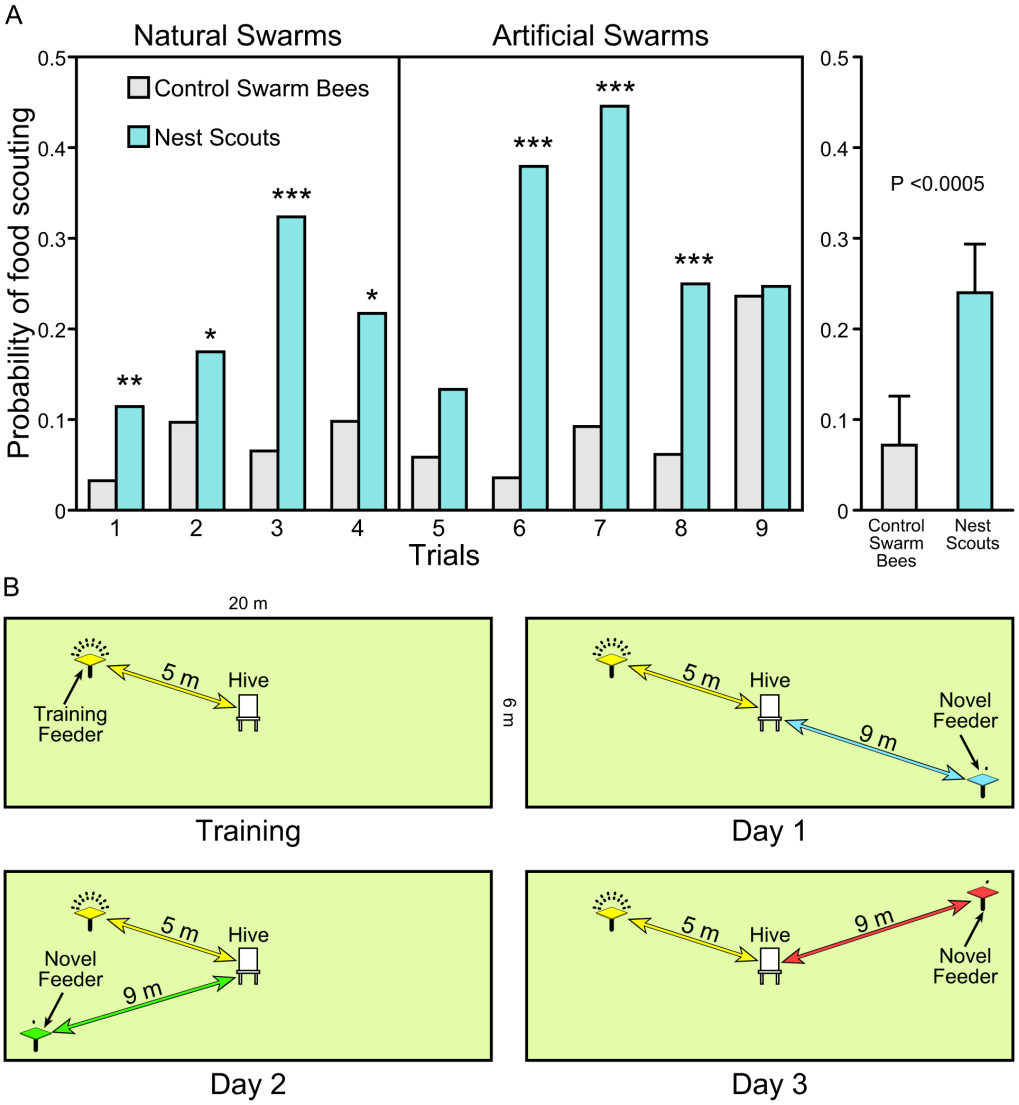

Figure 2.2 Transcriptomic analyses of individual differences in novelty seeking between food scouts (S) and non-scouts (NS) (n = 20 bees/group). **(A)** Selected microarray results highlight differences in brain expression for ten dopamine, octopamine, glutamate, or GABA signaling genes related to novelty seeking, motivation, and reward in vertebrates. *DopR2* and *OctR1* did not show significant differences in expression (in the latter case, probably because of very low expression levels). GABA transporter 1A gene (*Gat-a*) expression was one of the best correlates of scouting behavior (permutation *t*-test, $p < 0.05$). **(B)** Results of Linear Discriminant Analysis (LDA) for genes shown in Fig 2.2A demonstrate clear separation between most scouts and non-scouts based on differences in brain gene expression (standardized expression values: mean = 0, SD = 1). This plot of LD1 versus LD2 accounted for 82% of the variation in brain gene expression across scouts and non-scouts (n = 20 bees/group). S1, S2, S3 and N1, N2, N3: scouts and non-scouts, respectively, from 3 different colonies.

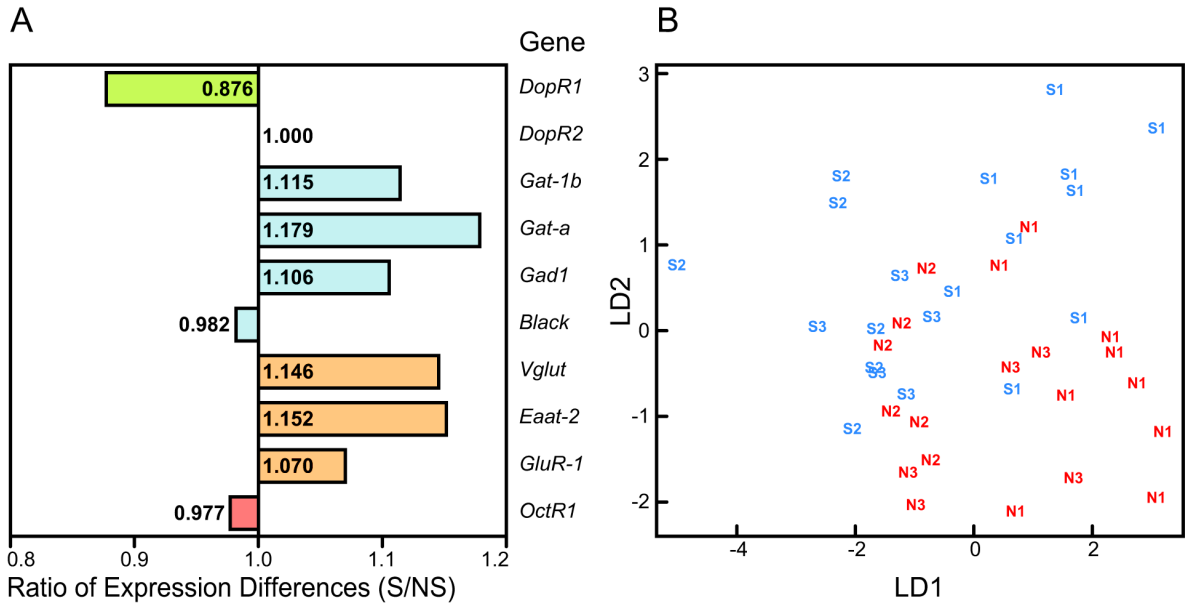

Figure 2.3 Glutamate or octopamine treatment increased the probability of scouting, while dopamine antagonist treatment decreased it (*p <0.05, ***p <0.0001). **(A)** Oral administration of monosodium glutamate (MSG) to non-scouts in sugar syrup (20 mg/ml) caused a significant effect in 7 out of 12 trials (with 11 colonies) over two years, an overall 73% increase in scouting probability compared to sucrose-fed-only control bees (p < 0.0001, mixed-model ANOVA, 2-tailed test). Octopamine (OA) treatment (4 mg/ml) caused a significant effect in 3 out of 10 trials (9 colonies, the same ones used for MSG) over two years, an overall 37% increase in scouting probability (p < 0.05). Statistical tests were performed on square-root transformed data; graph represents the untransformed mean ± SE of 12 trials for MSG (with 11 colonies) and 10 trials for octopamine (with 9 colonies); results of individual trials are shown in Figs. 2.6 and 2.7. **(B)** The glutamate vesicular transporter blocker Chicago Sky Blue (CSB) (4 mg/ml) blocked the effect of MSG on scouting (p<0.05, least-square mean ± SE for 4 previously MSG-responsive colonies; results of individual trials are shown in Fig 2.6). **(C)** Non-scout foragers treated with dopamine antagonists (either the D1-receptor antagonist SCH-23390, the "pan-receptor" antagonist Flupenthixol, or both), showed an overall 44% decrease in scouting probability in 7 trials over 3 colonies (p <0.05, graph represents least-square mean ± estimated error; mixed-model ANOVA, 2-tailed test; results of individual trials are shown in Fig 2.8). The probability of scouting was calculated from the proportion of foragers in each treatment group that exhibited scouting behavior, based on a precise count of foragers when releasing them from treatment cages.

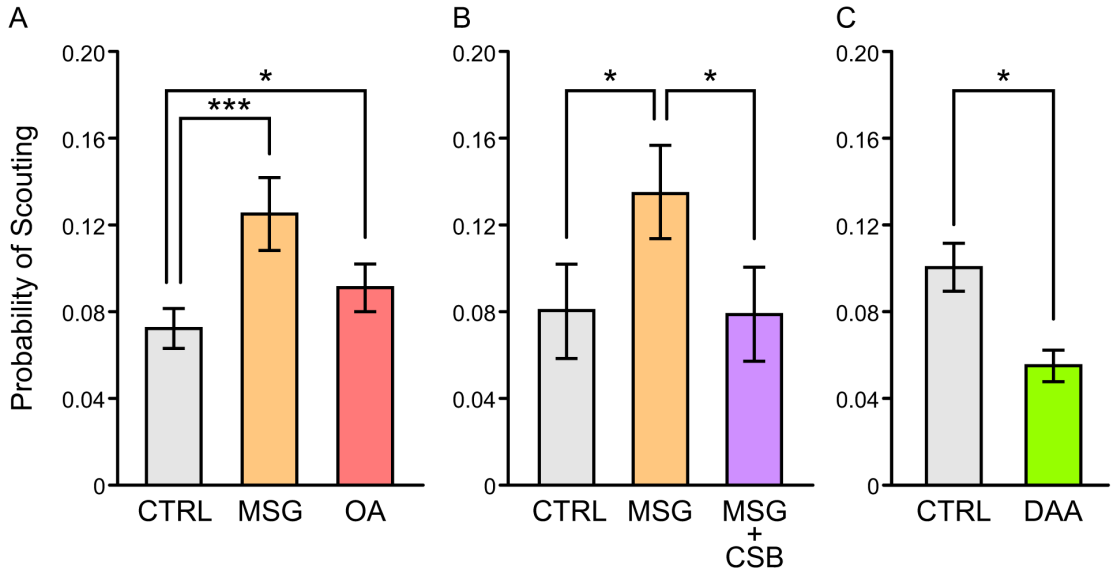

Figure 2.4 Scouts and non-scouts show differences in brain expression for genes related to novelty seeking, motivation and reward in vertebrates. **A)** RT-qPCR confirmation of microarray results of genes in glutamate or GABA signaling pathways. The same samples were used as in the microarray analysis (n = 18-20 bees/ behavior group, all from the Year 2 sample set). Least square means ± standard errors, and *p*-values for the behavior groups are shown (two-way ANOVA, * $p < 0.05$, ** $p < 0.01$, 2-tailed test). General patterns of all genes were the same as in microarrays. Up-regulation of *Eaat2*, *Vglut*, *Glu-RI* and *Gat-a* genes was confirmed. **B)** RT-qPCR results using both microarray samples (Year 2) and a different collection of samples (Year 1) confirmed the down-regulation of *DopR1* and detected 3 additional differentially expressed catecholamine receptor genes (shown here; n = 27-29 bees/group for Year 1, n = 19-20 bees/group for Year 2). Two collections were sampled from 6 different colonies (3 colonies each year, all SDI colonies).

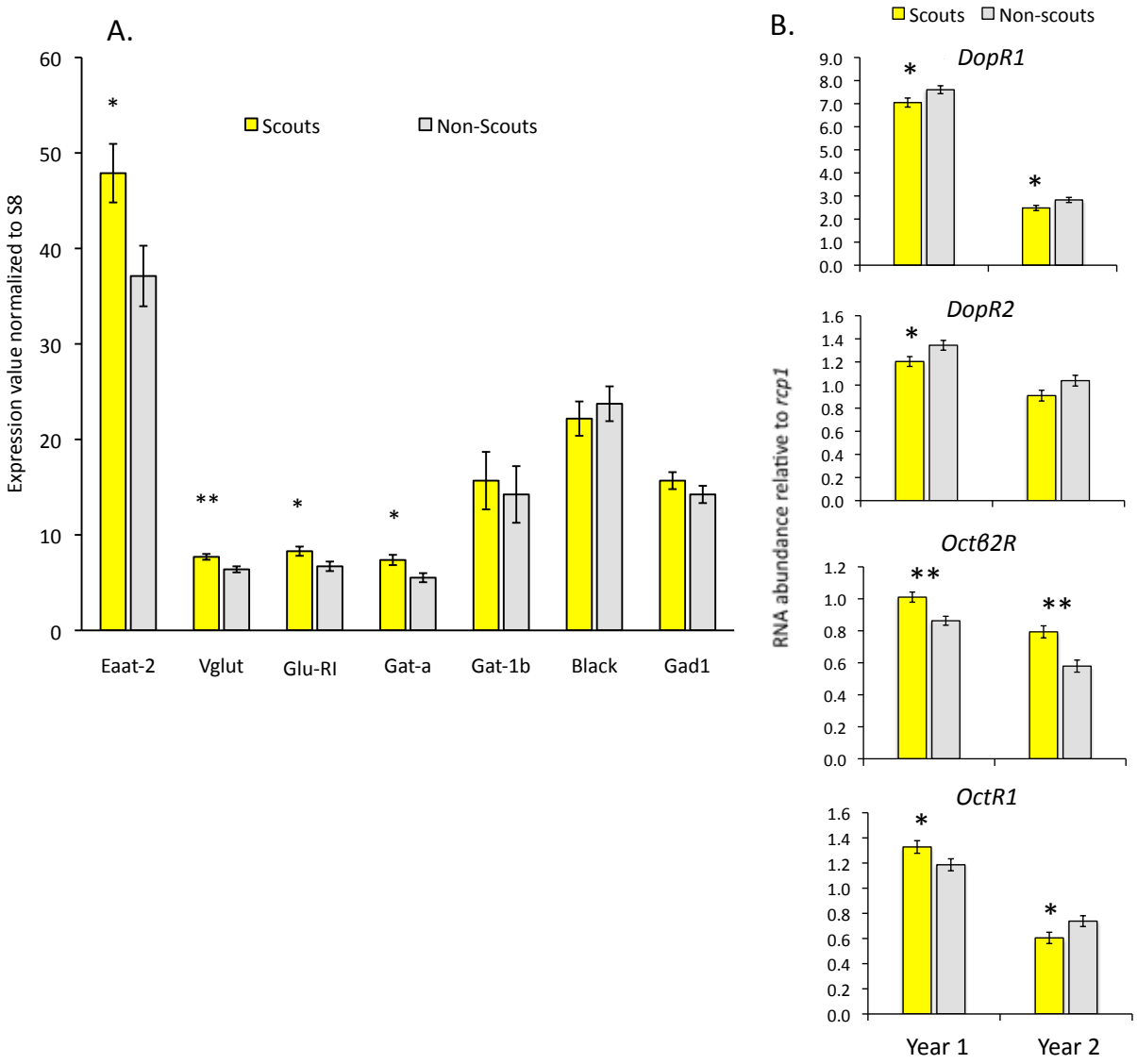

Figure 2.5 Similarities between food scouts identified via feeder-discovery and hive-moving assays. **(A)** Results of Hierarchical Clustering Analysis revealed strong clustering of scout and non-scout brain expression values for 10 neural signaling genes regardless of method of scout identification. Heatmap showing mean expression values for scouts and non-scouts identified with the feeder-discovery assay (S1 and N1, n = 20 bees /each group) or hive-moving assay (S2 and N2, n = 28-29 bees/group), for the 10 neural signaling genes. Expression values were standardized separately for each collection method (mean = 1, SD = 0). *DopR1*: D1-type dopamine receptor 1 gene; *DopR2*: D1-type dopamine receptor 2 gene; *OctR1*: α-adrenergic-type octopamine receptor 1 gene; *Gat-a* and *Gat-1b*: GABA neurotransmitter transporter genes A and 1B; *Glu-RI*: AMPA-type glutamate receptor I gene; *Eaat-2*: Excitatory amino acid transporter 2 gene; *Gad1*: glutamate decarboxylase 1 gene; *Vglut*: Vesicular glutamate transporter gene. *Black*: a putative glutamate decarboxylase 2 gene. **(B)** Results of Linear Discriminant Analysis (LDA) revealed strong clustering of scout brain expression values for these same genes regardless of method of scout identification. Red: scouts; Blue: non-scouts; same notation used in panel A.

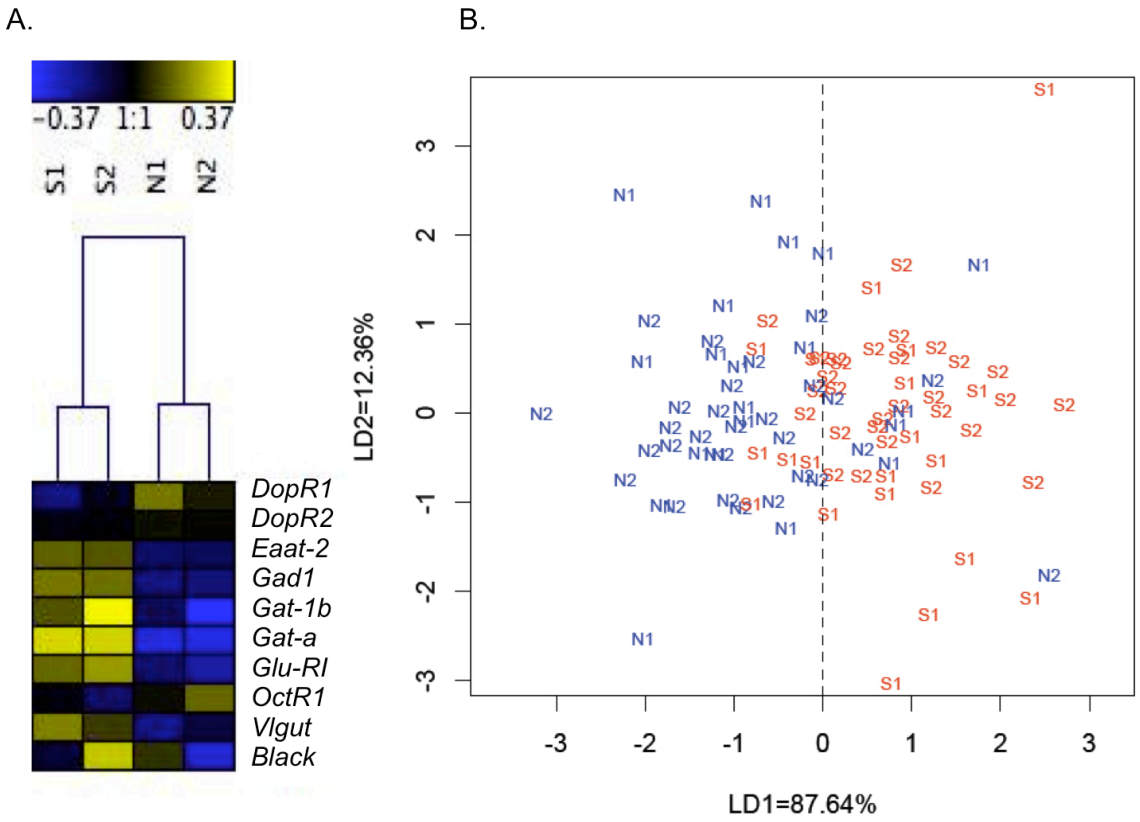

Figure 2.6 Effects of MSG and glutamate antagonist (CSB) on scouting probability, shown trial by trial. One colony was used for each trial (sometimes different treatment groups were used in different colonies). X-axis: trials named as "colony_date" and ordered by experiment date. Scouting probability P = n (Foragers that scouted) / n (Total foragers available) × 100%. Blue: control group; Orange: MSG-treated group, dose = 20 mg/ml. Purple: MSG+CSB-treated groups, dose = 20 mg/ml MSG + 4 mg/ml CSB. Statistical analysis: Fisher's Exact Test, two-tailed (except for trial B14_Sep 6, the last trial, which was one-tailed, based on the prior trials) * $p < 0.05$, ** $p < 0.01$. Sample sizes: n = 143-236 treated foragers/ trial, n = 101-239 control foragers/ trial, across all trials.

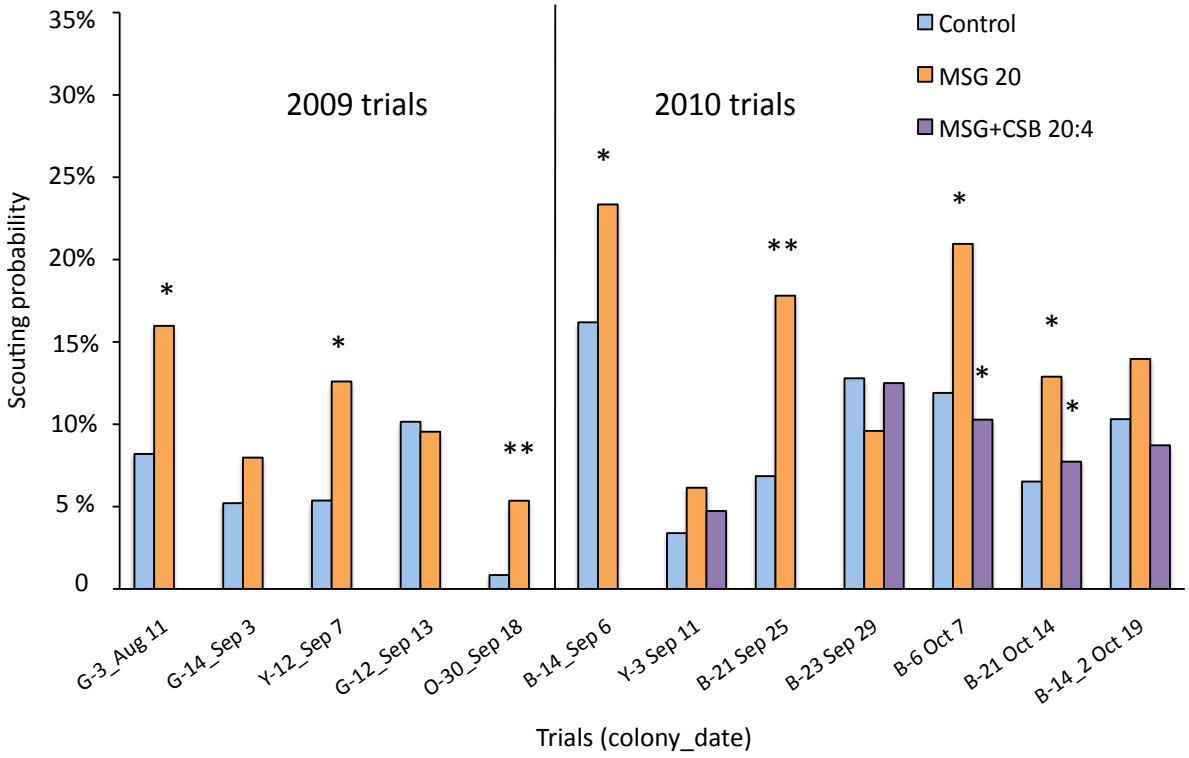

Figure 2.7 Effects of octopamine (OA) on scouting probability, shown trial by trial. Blue: control group; Red: OA-treated group, dose = 4 mg/ml. Sample sizes: n = 166-242 treated foragers/ trial, n = 101-241 control foragers/ trial. Notation and statistical analysis was described in Fig 2.6.

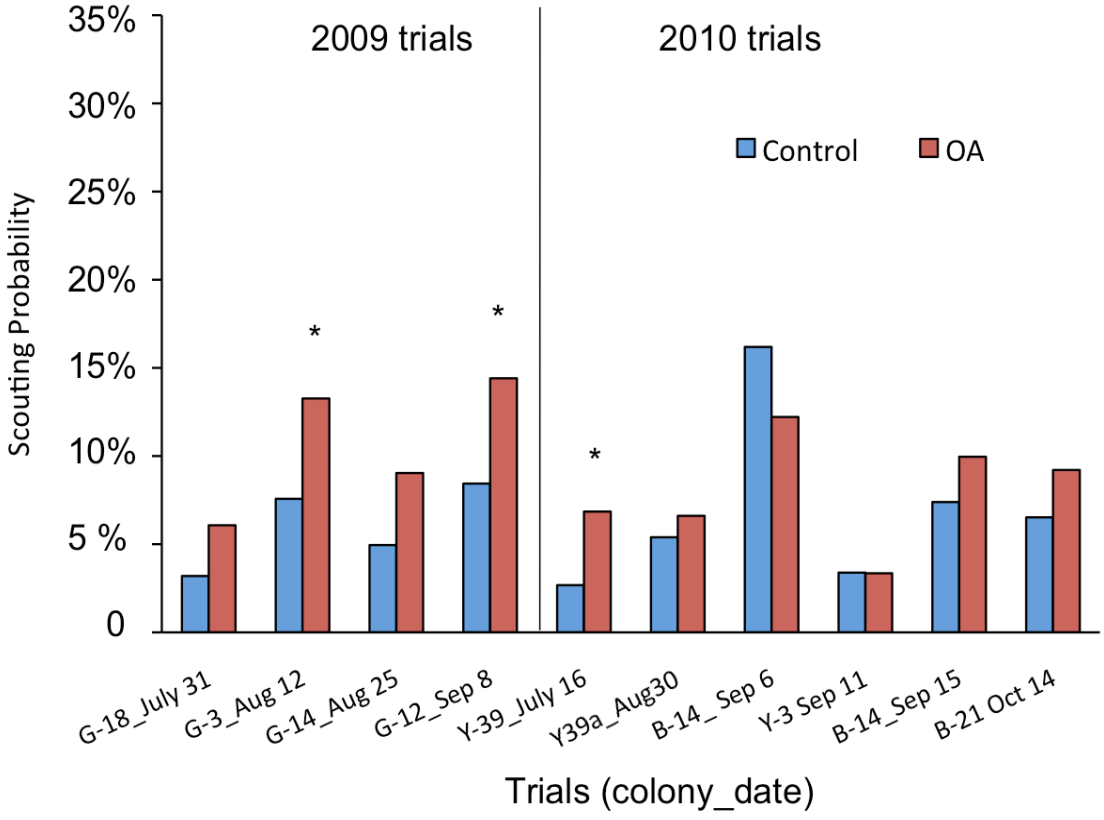

Figure 2.8 Effects of dopamine antagonists on scouting probability, alone or combined, shown trial by trial (colonies = 3). The pan-receptor antagonist Flupenthixol (FLUX) and/or the D1-specific antagonist SCH-23390 (SCH) were applied. Three trials using bees from Colony 2 were compared to the same (Colony 2) control group, and likewise for Colony 3. Statistical analysis: Fisher's Exact Test, two-tailed, * $p < 0.05$. Sample sizes: n = 85-150 treated foragers/ trial, n = 147-157 control foragers/ trial.

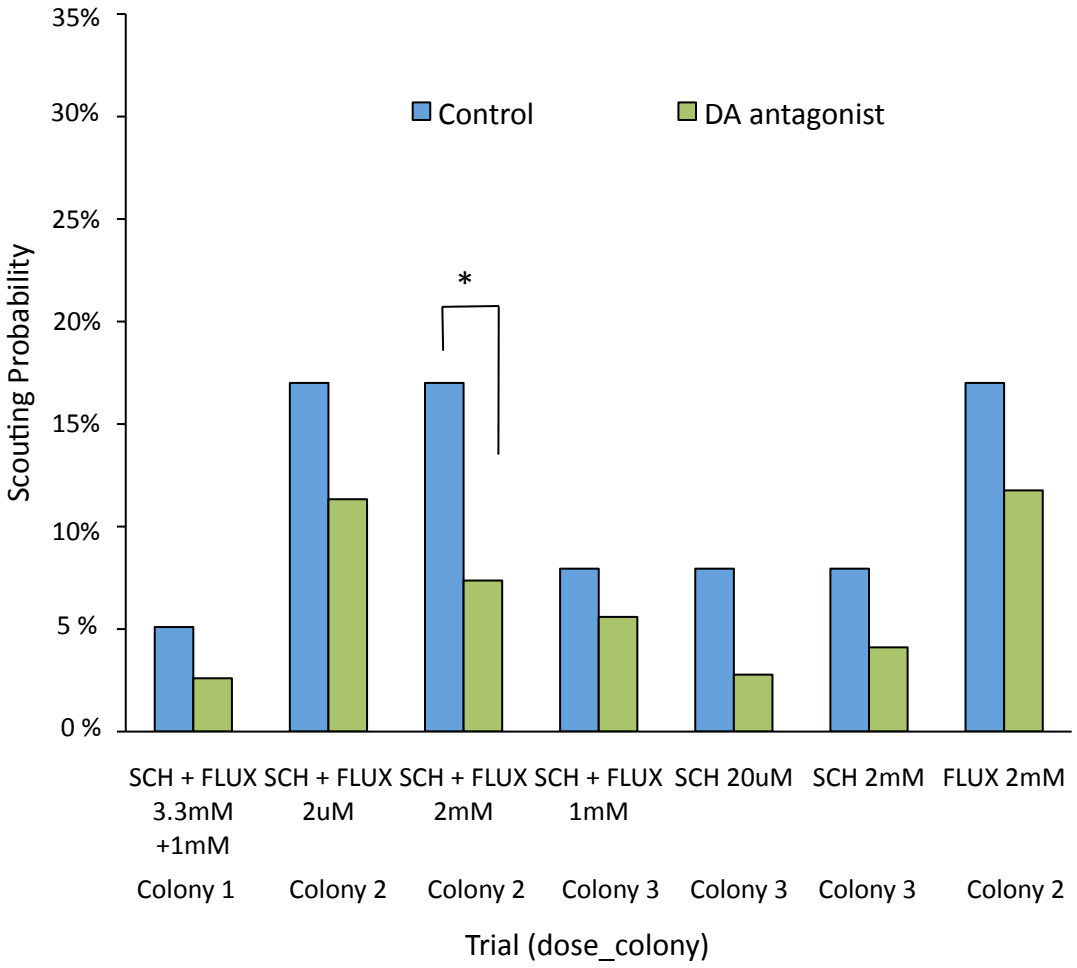

Figure 2.9 Effects of OA and MSG on non-scouting foraging activity. With recruiting allowed, there was a significant decrease in foraging activity in both MSG- and OA-treated groups. This result suggests that the treatment effects on scouting (obtained when recruiting was not allowed) were unlikely to be caused by general increases in foraging activity. Light blue: control group; yellow: MSG-treated group 20 mg/ml; Teal blue: OA-treated group 4 mg/ml. Sample sizes: n = 165-223 treated non-scouts/ trial, n = 149-195 control non-scouts/trial. Notation and statistical analysis was described in Fig 2.6.

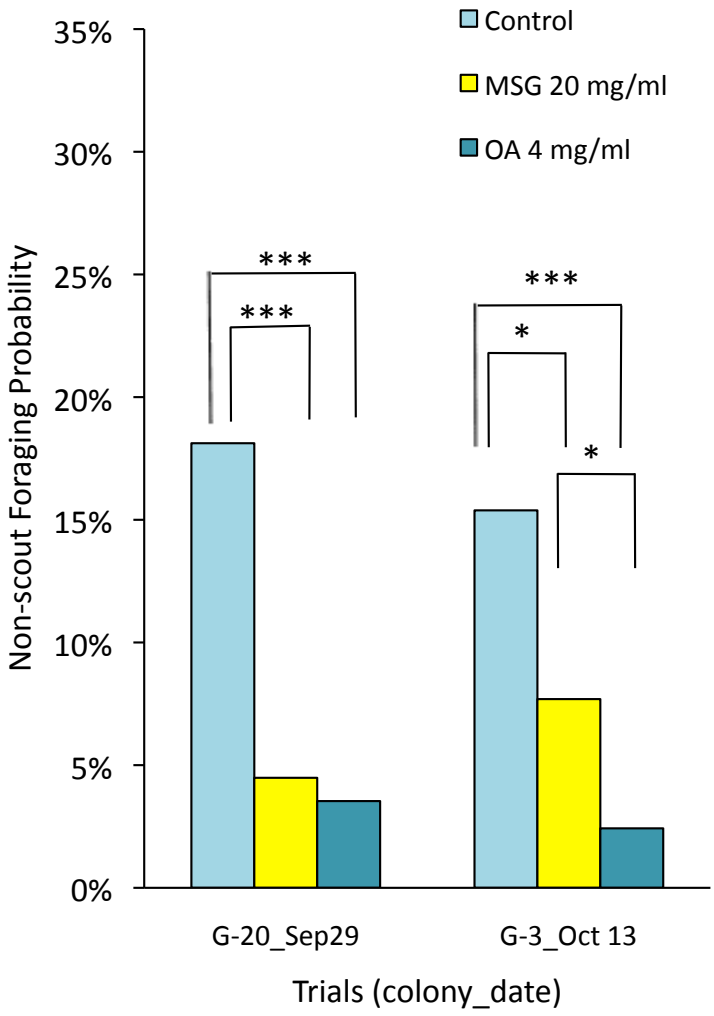

Figure 2.10 Results of dose response tests for OA and MSG. In these limited numbers of tests, no effect was observed on scouting probability for a lower dose of OA than reported in the main text, or for either a lower or higher dose of MSG. Blue: control group; Orange: MSG-treated group 10 mg/ml; Green: MSG-treated group 100 mg/ml Purple: OA-treated group 2 mg/ml. Sample sizes: n = 134-219 treated foragers/ trial, n = 116-221 control foragers/ trial.

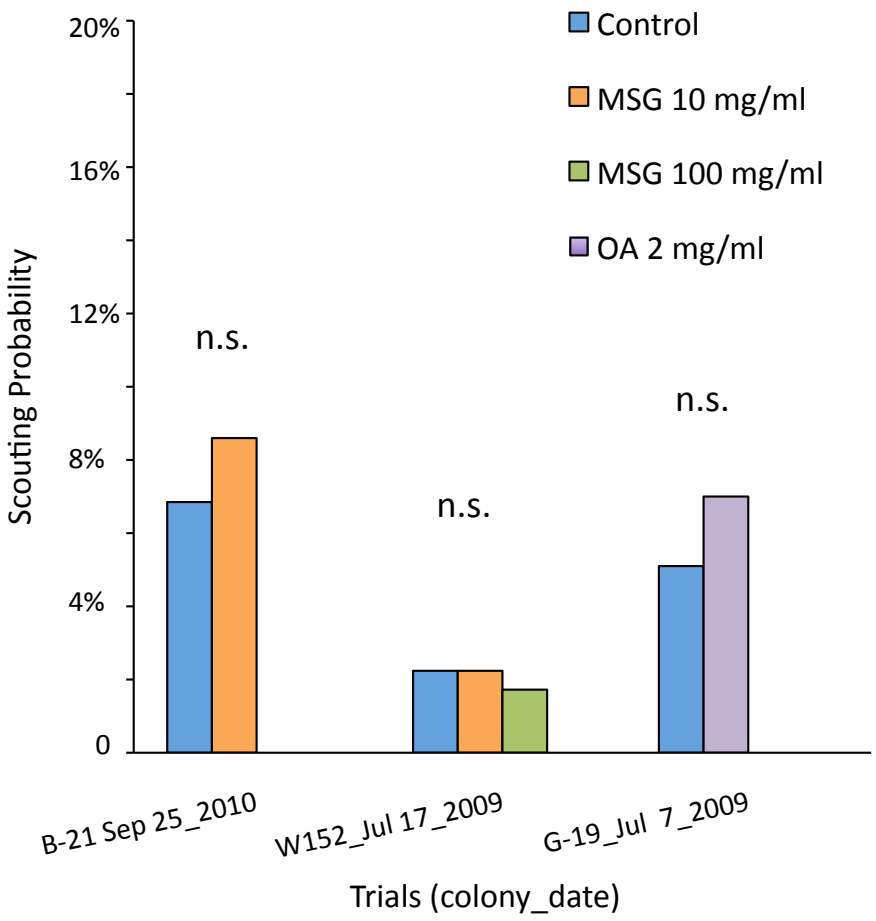

Figure 2.11 No effect on scouting probability for GABA or GABA receptor agonist treatments. Results for each trial were shown. Blue: control group; Black: GABA (20 mg/ml) or GABA receptor agonist TACA (5.88 or 6 mg/ml). Sample sizes: n = 139-209 treated foragers/ trial, n = 134-218 control foragers/ trial. Notation and statistical analysis was described in Fig 2.6.

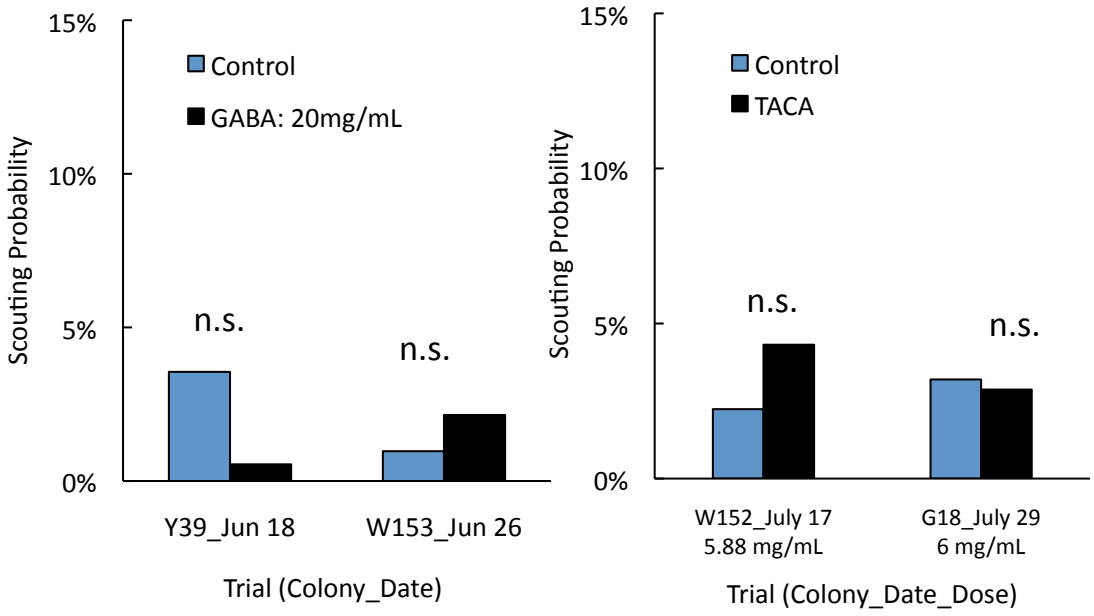

Table 2.1 Primer sequences for the genes analyzed by RT-qPCR. *DopR3, Dat, Nmdar1* were tested but were not differentially expressed in any experiments (data not shown). *Gene annotations were based on OGS 2.0 version of A. *mellifera* genome Assembly 4.0, last updated in April 2011. ** GB numbers were based on OGS 2.0 version of A. *mellifera* genome Assembly 4.0. ***Manual analysis showed the gene is a conserved ortholog of *Drosophila Glu-RI* (CG8842) gene. This annotation may differ from NCBI Genebank record (genomic locus: LOC411220); **** Genomic locus: LOC410752. (*) *DopR1* gene had a GB number "GB 30031_3" in Ame 4.0, but it is currently discontinued. The mRNA ID is NM_001011595.1 and available in NCBI.

Gene Name	*A.mellifera* Gene Annotation*	BeeBase GB Number**	FW primer	RV primer
DopR1	dopamine receptor 1	N/A (*)	ACAGAATTCCGAGAAGCGTTCA	ATTCGCTAGTCGACGGTTGATTT
DopR2	dopamine receptor 2	GB17921	ACACGGAATTGGTTCTCCATCT	TCCCGTAACCGGCTGTCA
DopR3	D2-like dopamine receptor	GB14561	GCGCGGAGGGAAATCTTAT	GGATTCTTACTGTGCGCTGTGT
OctR1	octopamine receptor 1	GB11266	ACGAAGGCGGCGAAGAC	CGCGCACCAAGTACATTGTG
OctR2	octopamine receptor 2	GB12240	CGTGTGCGTGTTCCAAGTG	GGGCAGCCAGAAACTGACA
Octβ2R	octopamine receptor beta-2R	GB18869	AGCGTTGGCCGACATGTT	AGCCATTTGCCGGTCAATT
Dat	dopamine transportor	GB15426	CGTATACCCTGTGTGGGCAAA	TTGCAATGCCGGGTATCAT
Nmdar1	NMDA-type glutamate receptor 1	GB19253	AACGGAAAACTTTGCGAGCTT	ACTCACGGTTGTAGGGTCGTTAA
Glu-RI***	similar to AMPA-type glutamate receptor I, CG8842	GB11443	GGGATCGCCTCATATACCCA	GAGCGAACCAAAGGCTGTTT
Eaat-2	excitatory amino acid transporter 2	GB16377	AATTTGACGGCAACTGCTCAG	GCGTGGAACAATAAGCCCAA
Vglut*****	similar to vesicular glutamate transporter CG9887	GB19507	GACACCTTGGCGGAAGTTCTT	TTCCAGGACCTGCAGAAATTG
Gat-a	GABA neurotransmitter transporter-1A	GB19372	GGGTTTATGGCTCATGAACAGC	CTAAGAAAGCCAATCCTGGGC
Gat-1b	GABA neurotransmitter transporter-1B	GB16752	GGTTGGTTTCATGGCTCATGA	AAGCTAAACCTGGACCGGAAG
Gad1	glutamate decarboxylase 1	GB15745	GCAAGGCCAAAGGACACATC	ATCGAACGCTCCAATGACG
Black	similar to black CG7811	GB19363	TCGAAATTGCAGATATTTGCGA	CAAACCACCTCCCCAAGCT

Table 2.2 Results of two-way ANOVA for RT-qPCR data in Fig 2.4 B. Group: 2 behavioral groups, scouts and non-scouts; Colony: different individual SDI colonies were used; G×C: Group by Colony Interaction. *df*: degree of freedom. Studentized Residue= ± 2.3 was used as cutoff for *post hoc* adjustment of outlier removal. Year 1: n = 27-29 bees/group. Year 2: n = 19-20 bees/group.

Year 1	**Least Square Means**		**Standard Error**		***p* (Group) *df* =1**	***p* (Colony) *df* = 2**	***p* (G×C) *df* =2**
Gene	**Scout**	**Non-scout**	**Scout**	**Non-scout**			
DopR1	7.047594	7.604234	0.195493	0.171281	**0.0372**	<0.0001	n.s
DopR2	1.203753	1.344501	0.042654	0.041732	**0.0222**	<0.0001	n.s
OctR1	1.327864	1.186317	0.050158	0.047387	**0.0358**	<0.0001	**0.0172**
Octb2R	1.010546	0.862890	0.031694	0.028185	**0.0010**	<0.0001	n.s
Year 2	**Least Square Means**		**Standard Error**		***p* (Group) *df* =1**	***p* (Colony) *df* = 2**	***p* (G×C) *df* =2**
Gene	**Scout**	**Non-scout**	**Scout**	**Non-scout**			
DopR1	2.480410	2.827646	0.112485	0.110885	**0.0350**	n.s	n.s
DopR2	0.908747	1.038117	0.046419	0.045758	0.0555	n.s	n.s
OctR1	0.604880	0.738132	0.044804	0.042878	**0.0390**	0.0023	n.s
Octb2R	0.793619	0.579244	0.037717	0.037717	**0.0003**	<0.0001	**<0.0001**

Table 2.3 Top 25 genes ranked by fold change (FC) (FDR<0.01). Repetitive probes were removed from the analysis. ABS.FC is the non-log-transformed fold change for both up- and down-regulated genes. Yellow: up-regulated FC= Scout/Non-scouts; Blue: down-regulated FC= Non-scouts/Scouts.

GB number	FDR	ABS. FC	Gene Annotation	Fly Ortholog
XR_0149	2.00E-07	1.5468326	Apis mellifera hypothetical protein LOC727513 (LOC727513), partial mRNA	n/a
GB13587	5.49E-12	1.4580348	Apis mellifera odorant binding protein ASP4 (LOC406101)	n/a
GB16215	1.03E-07	1.3713854	Apis mellifera similar to Myosin alkali light chain 1 CG5596-PA, isoform A, transcript variant 4 (LOC410058)	CG5596
DB75735	1.32E-06	1.3564271	DB757352 RIKEN full-length enriched honey bee cDNA library, head Apis mellifera cDNA clone BH10009C23 3'	n/a
GB13236	5.22E-03	1.3108676	Apis mellifera similar to CG5958-PA (LOC552447)	CG5958
GB16807	7.60E-03	1.2788393	Apis mellifera similar to CG9518-PA (LOC410733)	n/a
BP87455	8.46E-05	1.2757671	BP874552 adult brain Apis mellifera cDNA clone p36E07_E07_09.ab1	n/a
GB10517	1.99E-05	1.2744674	Apis mellifera similar to juvenile hormone acid methyltransferase CG17330-PA (LOC724216)	CG17330
NW_0012	1.66E-03	1.2613549	NW_001253491.1 SET: UI_EST BI504108; linkage group 7 genomic contig (based on Amel_4.0 Group7.45)	n/a
GB14621	7.25E-10	1.2609549	Apis mellifera similar to Protein lethal(2)essential for life (Protein Efl21) (LOC724488)	CG4533
BB16001	1.48E-04	1.2594896	BB160011B20D05.5 Bee Brain Normalized Library, BB16 Apis mellifera cDNA clone BB160011B20D05 5'	n/a
GB18662	1.48E-04	1.2389366	Apis mellifera similar to Protein lethal(2)essential for life (Protein Efl21), transcript variant 1 (LOC410087)	n/a
AM12832	7.41E-05	1.2361189	AM12832 Unknown [Arrayset: JDE_EST jdeC12_, jdeC12_ GroupUn.5108_836_905]	n/a
GB13186	2.24E-03	1.2361139	Apis mellifera similar to WW45 protein (LOC552413)	CG33193
GB18819	1.48E-04	1.2270127	antennal-specific protein 3c precursor (Asp3c)	CG11390
GB11462	1.45E-03	1.2194592	GB11462-RA	CG5183
GB11118	2.11E-04	1.2172083	Apis mellifera similar to cell-cycle related and expression-elevated protein in tumor (LOC412928)	CG9018
GB14695	6.77E-03	1.2169004	Apis mellifera similar to Rabphilin CG11556-PA (LOC413784)	CG11556
DB76836	7.23E-04	1.2165799	DB768367 RIKEN full-length enriched honey bee cDNA library, head Apis mellifera cDNA clone BH10048A20 3'	n/a
BB17002	4.80E-05	1.2099707	BB170020A20F01.5 Bee Brain Normalized/Subtracted Library, BB17 Apis mellifera cDNA clone BB170020A20F01 5'	n/a
GB15018	7.00E-05	1.2098201	Apis mellifera similar to B0238.12 (LOC725380)	n/a
GB16890	2.96E-03	1.2073442	Apis mellifera similar to mitochondrial solute carrier-like protein, transcript variant 2 (LOC552801)	CG4963
GB14435	3.97E-06	1.2071627	Apis mellifera similar to Protein lethal(2)essential for life (Protein Efl21) (LOC724367)	n/a
GB10483	2.25E-03	1.2066759	Apis mellifera similar to CG9451-PA (LOC726737)	n/a
NW_0012	7.00E-03	1.2059346	NW_001253288.1 SET: JDE_EST jdeF08_3DEF1; linkage group 2 genomic contig (based on Amel_4.0 Group2.12)	n/a

Table 2.4 List of 1219 differentially expressed transcripts between scouts and non-scouts (FDR<0.05). Table S3B was included as Appendix A.

Table 2.5 Gene Ontology functional analysis was performed using 670 genes with high-confidence orthologs to genes in *Drosophila melanogaster* (circa May 2009), out of 1219 differentially regulated transcripts (FDR<0.05). Overrepresented GO terms in up- and down-regulated genes are shown (broadest or redundant terms removed in GO FAT). Enrichment analysis performed with both Fisher's Exact Test with *p*-value <0.01 (all terms in the table) and more stringent Benjamini hypergeometric test with *p*-value <0.05 (yellow highlighted terms). Six KEGG pathways were identified among these enriched GO terms.

Category	Term	EASE P-Value	Fold Enrichment	Benjamini
Biological Process				
GOTERM_BP_FAT	GO:0006366~transcription from RNA polymerase II promoter	1.03E-05	2.49	1.53E-02
GOTERM_BP_FAT	GO:0000022~mitotic spindle elongation	1.50E-04	2.31	1.06E-01
GOTERM_BP_FAT	GO:0051231~spindle elongation	1.50E-04	2.31	1.06E-01
GOTERM_BP_FAT	GO:0032774~RNA biosynthetic process	3.84E-04	2.06	1.75E-01
GOTERM_BP_FAT	GO:0006351~transcription, DNA-dependent	3.84E-04	2.06	1.75E-01
GOTERM_BP_FAT	GO:0006412~translation	4.88E-04	1.53	1.67E-01
GOTERM_BP_FAT	GO:0006350~transcription	1.56E-03	1.54	3.73E-01
GOTERM_BP_FAT	GO:0022900~electron transport chain	6.71E-03	1.89	8.13E-01
GOTERM_BP_FAT	GO:0006091~generation of precursor metabolites and energy	8.96E-03	1.55	8.54E-01
GOTERM_BP_FAT	GO:0006120~mitochondrial electron transport, NADH to ubiquinone	1.25E-02	2.31	9.05E-01
GOTERM_BP_FAT	GO:0045333~cellular respiration	1.33E-02	1.75	8.92E-01
GOTERM_BP_FAT	GO:0048515~spermatid differentiation	1.61E-02	2.36	9.12E-01
GOTERM_BP_FAT	GO:0007286~spermatid development	1.61E-02	2.36	9.12E-01
GOTERM_BP_FAT	GO:0006119~oxidative phosphorylation	1.83E-02	1.64	9.19E-01
GOTERM_BP_FAT	GO:0042775~mitochondrial ATP synthesis coupled electron transport	1.83E-02	1.89	9.01E-01
GOTERM_BP_FAT	GO:0007052~mitotic spindle organization	2.11E-02	1.51	9.15E-01
GOTERM_BP_FAT	GO:0042773~ATP synthesis coupled electron transport	2.23E-02	1.85	9.10E-01
GOTERM_BP_FAT	GO:0006352~transcription initiation	2.62E-02	2.00	9.29E-01
GOTERM_BP_FAT	GO:0006367~transcription initiation from RNA polymerase II promoter	2.62E-02	2.00	9.29E-01
GOTERM_BP_FAT	GO:0015980~energy derivation by oxidation of organic compounds	2.90E-02	1.62	9.36E-01
GOTERM_BP_FAT	GO:0022904~respiratory electron transport chain	3.19E-02	1.77	9.43E-01
GOTERM_BP_FAT	GO:0043933~macromolecular complex subunit organization	3.94E-02	1.38	9.65E-01
GOTERM_BP_FAT	GO:0042063~gliogenesis	4.63E-02	2.13	9.76E-01
GOTERM_CC_FAT	GO:0033279~ribosomal subunit	5.97E-08	2.07	1.97E-05
GOTERM_CC_FAT	GO:0005840~ribosome	4.30E-07	1.95	7.11E-05
GOTERM_CC_FAT	GO:0022626~cytosolic ribosome	4.68E-07	2.48	5.17E-05
GOTERM_CC_FAT	GO:0044445~cytosolic part	1.18E-06	2.22	9.76E-05
GOTERM_CC_FAT	GO:0015934~large ribosomal subunit	1.72E-05	2.11	1.14E-03
GOTERM_CC_FAT	GO:0030529~ribonucleoprotein complex	1.89E-05	1.58	1.04E-03
GOTERM_CC_FAT	GO:0022625~cytosolic large ribosomal subunit	3.71E-05	2.61	1.75E-03
GOTERM_CC_FAT	GO:0070013~intracellular organelle lumen	8.66E-05	1.47	3.58E-03
GOTERM_CC_FAT	GO:0043233~organelle lumen	8.66E-05	1.47	3.58E-03
GOTERM_CC_FAT	GO:0016591~DNA-directed RNA polymerase II, holoenzyme	1.49E-04	2.87	5.45E-03
GOTERM_CC_FAT	GO:0015935~small ribosomal subunit	1.73E-04	2.26	5.72E-03
GOTERM_CC_FAT	GO:0031974~membrane-enclosed lumen	2.74E-04	1.42	8.21E-03
GOTERM_CC_FAT	GO:0044451~nucleoplasm part	3.91E-04	1.72	1.07E-02
GOTERM_CC_FAT	GO:0005654~nucleoplasm	1.50E-03	1.61	3.75E-02
GOTERM_CC_FAT	GO:0005665~DNA-directed RNA polymerase II, core complex	2.02E-03	4.16	4.68E-02
GOTERM_CC_FAT	GO:0043232~intracellular non-membrane-bounded organelle	2.40E-03	1.29	5.17E-02
GOTERM_CC_FAT	GO:0043228~non-membrane-bounded organelle	2.40E-03	1.29	5.17E-02
GOTERM_CC_FAT	GO:0031981~nuclear lumen	2.68E-03	1.49	5.40E-02
GOTERM_CC_FAT	GO:0022627~cytosolic small ribosomal subunit	5.96E-03	2.38	1.10E-01
GOTERM_CC_FAT	GO:0005829~cytosol	6.88E-03	1.43	1.19E-01
GOTERM_CC_FAT	GO:0030964~NADH dehydrogenase complex	1.47E-02	2.14	2.27E-01
GOTERM_CC_FAT	GO:0005747~mitochondrial respiratory chain complex I	1.47E-02	2.14	2.27E-01
GOTERM_CC_FAT	GO:0045271~respiratory chain complex I	1.47E-02	2.14	2.27E-01
GOTERM_CC_FAT	GO:0070469~respiratory chain	1.48E-02	1.78	2.18E-01

Table 2.5 (cont.)

Category	Term	EASE P-Value	Fold Enrichment	Benjamini
Biological Process				
GOTERM_CC_FAT	GO:0055029~nuclear DNA-directed RNA polymerase complex	1.79E-02	2.68	2.47E-01
GOTERM_CC_FAT	GO:0000428~DNA-directed RNA polymerase complex	1.79E-02	2.68	2.47E-01
GOTERM_CC_FAT	GO:0030880~RNA polymerase complex	1.79E-02	2.68	2.47E-01
GOTERM_CC_FAT	GO:0005739~mitochondrion	2.03E-02	1.24	2.66E-01
GOTERM_CC_FAT	GO:0005811~lipid particle	2.16E-02	1.35	2.70E-01
GOTERM_CC_FAT	GO:0005746~mitochondrial respiratory chain	2.22E-02	1.75	2.66E-01
GOTERM_CC_FAT	GO:0044429~mitochondrial part	2.57E-02	1.27	2.91E-01
GOTERM_CC_FAT	GO:0044455~mitochondrial membrane part	2.83E-02	1.53	3.07E-01
GOTERM_CC_FAT	GO:0005667~transcription factor complex	3.03E-02	1.88	3.14E-01
GOTERM_CC_FAT	GO:0000314~organellar small ribosomal subunit	3.85E-02	2.06	3.72E-01
GOTERM_CC_FAT	GO:0005763~mitochondrial small ribosomal subunit	3.85E-02	2.06	3.72E-01
GOTERM_CC_FAT	GO:0000313~organellar ribosome	3.86E-02	1.59	3.62E-01
GOTERM_CC_FAT	GO:0005761~mitochondrial ribosome	3.86E-02	1.59	3.62E-01
GOTERM_CC_FAT	GO:0031980~mitochondrial lumen	4.63E-02	1.40	4.07E-01
GOTERM_CC_FAT	GO:0005759~mitochondrial matrix	4.63E-02	1.40	4.07E-01
GOTERM_CC_FAT	GO:0005675~holo TFIIH complex	4.76E-02	4.28	4.06E-01
GOTERM_MF_FAT	GO:0003735~structural constituent of ribosome	9.45E-09	2.23	5.63E-06
GOTERM_MF_FAT	GO:0005198~structural molecule activity	9.73E-07	1.85	2.90E-04
GOTERM_MF_FAT	GO:0003899~DNA-directed RNA polymerase activity	3.61E-03	3.13	5.12E-01
GOTERM_MF_FAT	GO:0034062~RNA polymerase activity	3.61E-03	3.13	5.12E-01
GOTERM_MF_FAT	GO:0003954~NADH dehydrogenase activity	5.22E-03	2.45	5.41E-01
GOTERM_MF_FAT	GO:0008137~NADH dehydrogenase (ubiquinone) activity	8.96E-03	2.57	6.58E-01
GOTERM_MF_FAT	GO:0016655~oxidoreductase activity, acting on NADH or NADPH, quinone	8.96E-03	2.57	6.58E-01
GOTERM_MF_FAT	GO:0050136~NADH dehydrogenase (quinone) activity	8.96E-03	2.57	6.58E-01
GOTERM_MF_FAT	GO:0016651~oxidoreductase activity, acting on NADH or NADPH	1.16E-02	2.14	6.86E-01
GOTERM_MF_FAT	GO:0016251~general RNA polymerase II transcription factor activity	1.44E-02	1.89	7.09E-01
GOTERM_MF_FAT	GO:0016667~oxidoreductase activity, acting on sulfur group of donors	2.15E-02	2.63	8.01E-01
GOTERM_MF_FAT	GO:0004091~carboxylesterase activity	2.87E-02	2.32	8.54E-01
GOTERM_MF_FAT	GO:0004540~ribonuclease activity	3.69E-02	2.22	8.94E-01
GOTERM_MF_FAT	GO:0003714~transcription corepressor activity	4.95E-02	3.29	9.36E-01
INTERPRO	IPR009072:Histone-fold	6.62E-03	3.18	9.99E-01
INTERPRO	IPR012677:Nucleotide-binding, alpha-beta plait	1.15E-02	1.85	9.97E-01
INTERPRO	IPR000504:RNA recognition motif, RNP-1	1.37E-02	1.82	9.90E-01
KEGG_PATHWAY	dme03010:Ribosome	2.52E-08	2.71	1.89E-06
KEGG_PATHWAY	dme03020:RNA polymerase	2.75E-03	3.19	9.80E-02
KEGG_PATHWAY	dme00190:Oxidative phosphorylation	5.05E-03	1.71	1.19E-01
KEGG_PATHWAY	dme00240:Pyrimidine metabolism	8.48E-03	2.03	1.48E-01
KEGG_PATHWAY	dme03018:RNA degradation	2.53E-02	2.08	3.19E-01
KEGG_PATHWAY	dme03420:Nucleotide excision repair	3.46E-02	2.39	3.56E-01
SMART	SM00360:RRM (RNA recognition motif)	2.79E-03	2.09	3.73E-01
SP_PIR_KEYWORDS	ribosomal protein	7.38E-08	2.21	1.96E-05
SP_PIR_KEYWORDS	ribonucleoprotein	1.30E-05	2.11	1.72E-03
SP_PIR_KEYWORDS	acetylation	1.23E-02	3.24	6.64E-01
SP_PIR_KEYWORDS	Transcription	1.25E-02	1.52	5.64E-01
SP_PIR_KEYWORDS	ribosome	1.99E-02	2.67	6.56E-01
SP_PIR_KEYWORDS	nucleus	3.37E-02	1.25	7.80E-01

Table 2.6 Percentages of scouts that returned to the hive with nectar or pollen in the pharmacology experiments. No significant differences were found between control and treated groups. There also was no significant difference between the two control groups (U-test, p=0.8357) and the two treated groups (MSG vs. OA) (U-test, p=0.2949).

Treatment Group	Percentage of Scouts Returning with Nectar/Pollen (Mean ± SD)	Number of Trials	Mixed-model ANOVA (2-tailed test)	Mann-Whitney U-test (2-tailed test)
Control	43.5 ± 26.1% (137 bees)	7	p = 0.3586	p = 0.3176
MSG-treated	55.3 ± 18.1% (226 bees)			
Control	48.9 ± 31.5% (91 bees)	6	p = 0.6854	p = 0.2774
OA-treated	41.5 ± 21.8% (109 bees)			

Table 2.7 Effect of pharmacological treatments on average food consumption and survival rates. Data are from the same colonies used for behavioral analyses in Fig 2.3 A, B, and C. Estimated consumption per bee was calculated by taking the total amount of sugar solution consumed by the caged bees and dividing it by the number that were alive at the end of the treatment.

Treatment	Dose	Average survival rate	Difference compared to control (t-test)	Estimated consumption per bee	Difference compared to control/MSG (t-test)
MSG	20 mg/ml	89.7%	n.s.	0.09 ml	$p < 0.05$
MSG+CSB	20 mg/ml; 4 mg/ml	81.7%	n.s.	0.06 ml	$p < 0.01$ (vs. control) $p = 0.08$ (vs. MSG, 4 colonies) $p = 0.02$ (vs. MSG 5 colonies)
Control	50% m/v	86.2%	---	0.11 ml	---
OA	4 mg/ml	93.6%	n.s.	0.09 ml	n.s.
Control	50% m/v	90.0%	---	0.09 ml	---
SCH or SCH+FLUX	2 uM -2 mM	70.4%	n.s.	0.06 ml	n.s.
Control	25-40% m/v	85.4%	---	0.07 ml	---

CHAPTER 3: COMPARATIVE BRAIN TRANSCRIPTOMIC ANALYSES OF NOVELTY SEEKING ACROSS DISTINCT BEHAVIORAL CONTEXTS IN HONEY BEES

ABSTRACT

Individual differences in behavior are often consistent across time and contexts, but it is not clear if such behavioral tendencies share the same molecular basis in different contexts. Honeybee scouts and recruits consistently differ in their tendency to seek novelty and do so in either foraging or nest hunting contexts. We compared the brain transcriptomes of scout bees searching for new food sources or new nest sites with those of recruits collected in the same contexts. Our findings revealed that the neurogenomic profiles of food and nest scouts significantly overlapped as did their functional enrichments, despite large expression differences associated with the two ecological contexts. Both expression profiles also significantly overlapped with that of food scouts collected in a controlled environment. The monoamine, glutamate, GABA and acetylcholine neurotransmitter systems were among a shared molecular signature between scouting behaviors in the two contexts, consistent with their shared novelty-seeking tendency as scouts. We identified a molecular signature of 89 genes that was sufficient to predict a bee's role as a scout or a recruit with 92.5 % success. Our discovery of a shared molecular signature of scouting behavior across different ecological contexts supports scouting tendency as an animal personality and contributes to the understanding of its evolution, especially in social species.

INTRODUCTION

Individual differences are an important feature of complex behavioral traits. Many such differences are heritable, relatively stable over the lifetime of an animal, and consistent across distinct behavioral contexts, in both humans [1, 2] and non-human animals [3, 4, 5]. Consistent individual behavioral differences in animals across time or contexts often are referred to as "behavioral syndromes" at the population level, or "animal personality" at the individual level [3, 4, 5]. This phenomenon exists in a wide range of species, from insects to primates [5, 6, 7] and also involves many different types of behavioral tendencies, including aggressiveness [8, 9], fearfulness [10], risk-taking [11,12,13] and exploratory or novelty-seeking behavior [14,15]. On the other hand, personality limits an individual's plasticity and prevents them from optimizing their behavioral response across environmental contexts and situations [16]. Individual differences in behavior pose both molecular and evolutionary questions, about 1) the underlying mechanisms of consistent behavioral tendencies, and 2) the adaptive value of limiting behavioral plasticity. Our experimental work focused on the molecular basis of individual behavioral variations in a social system [15,17] to search for insights that will help answer these unsolved, fascinating questions.

The scouting behavior of the honey bee, *Apis mellifera,* provides an excellent system to study the molecular basis of individual behavioral variations. Scouting behavior is an exploratory behavior that is performed by only a fraction of worker bees in two distinct ecological contexts: foraging and nest hunting. Scout bees search for new food resources or nest sites on their own, while recruit bees wait and rely on information provided by

scout bees to guide their foraging or nest site selections. The two scouting behaviors have very different search targets, evaluation criteria, and social environments that influence them [18]. Food scouts search for new flower patches and evaluate how profitable they are as sources of nectar and pollen. Nest scouts search for new dwellings such as tree holes and evaluate their volume, orientation, and location as proper nest sites [18]. Food and nest scouting are both critical processes for seeking new resources and are vital to colony survival, but they differ markedly in both behavior and the context in which they occur, for example, food scouting is a part of daily foraging activity and is performed during normal colony condition, while nest scouting only occurs one or twice a year and is involved during swarming when half of the bees leave the hive to establish a new colony [18].

Researchers have long speculated about whether there are links between the two types of scouting behaviors in honey bees, i.e., whether food scouts are more likely to also act as nest scouts or *vice versa* relative to other bees old enough to perform these tasks [17]. Lindauer [19] reported foraging activity typically ceases during nest hunting, suggesting a link between the two. We recently reported [15] that nest scouts are over three times more likely to act as food scouts, further supporting the idea of a link.

These consistent behavioral tendencies across contexts are hallmarks of animal personalities [5], but the molecular basis of such tendency in scouting remains elusive. Liang et al. [15] demonstrated that food scouts show large differences in brain gene expression relative to recruits. In addition, pharmacological experiments demonstrated a causal role for neural signaling pathways known to be involved in vertebrate novelty

seeking [15]. Together, these results strongly support the idea that scouting in honey bees is a personality trait, but molecular comparisons of individual nest and food scouts have not yet been performed.

If scouting in honey bees is a behavioral syndrome or personality trait, then we hypothesize there are common patterns of brain gene expression for both types of scouts, despite the differences in behavior and context. We would not necessarily expect identical patterns of brain gene expression; instead, we would expect a common core of genes to show similar patterns across both types of scouting, reflecting the shared novelty-seeking aspect of the different types of scouting behaviors. In this study, we tested this hypothesis using a honey bee whole genome microarray to gain an unbiased survey of brain gene expression. We compared scouts and recruits in two contexts: food scouts and food recruits during foraging, and nest scouts and nest recruits during nest hunting (Fig 3.1A).

MATERIAL AND METHODS

Bees

Bees were collected from four colonies maintained at Cornell University, Ithaca, New York. Each colony was headed by a queen that was instrumentally inseminated with a single, unrelated, male (SDI colonies) to minimize the effects of genetic variation within each trial (queens were reared and artificially inseminated at Glenn Apiaries, Fallbrook, CA). Three colonies were used to collect food scouts and recruits, and two colonies were used to collect nest scouts and recruits (one colony was used by both food and nest

scouting experiments). All bees were collected only in the morning to eliminate the circadian effect on brain gene expression.

Hive-moving assay for collecting food scouts and recruits.

Each colony used for identifying food scouts and recruits had its hive entrance screened and then was moved to a new location at least 2.5 km away in the evening. Hive entrances were opened on the following morning at 8.00, and scout collections took place for 1 hour. Scouts were identified as the first bees to leave the hive, forage in the unfamiliar environment, and return to their hive [20, 21]. To prevent recruitment, the hive entrance was screened so that foragers could leave but not return [22]. Analysis of foregut contents was performed to further verify scout status [20, 22]. Only scouts that carried a small amount of nectar were used. Food recruits were collected between 10.00-11.00 the next morning, which gave the colonies time to initiate foraging and recruitment that morning. Once again, the entrance was screened until 50 returning foragers were collected by immediately dropping them into liquid nitrogen. Foregut contents were checked to confirm that they had a full honey stomach, which is typical for recruited foragers.

Artificial swarming method for collecting nest scouts and recruits.

Artificial swarms were prepared according to standard procedures [23] during late May and early June. A different colony with a single-drone inseminated (SDI) queen was used in each artificial swarm trial. We first located the colony's queen and put her in a small cage (3.2×10×1.6 cm). Using a large funnel, we then shook 1.5 kg (~12,000) bees into a wooden "swarm box" (15×25×35 cm) with screen wire sides and placed the queen cage

inside it. To obtain both young bees and foragers, we shook bees off frames located in both the upper and lower parts of the hive. The swarm box was then placed in a dark room and kept at room temperature for 3-5 days. The screen sides were also brushed with sucrose syrup (1:1 v/v) about 5 times a day, until significant amounts of wax scales dropped off the bees. The artificial swarm was transferred outside in the morning at 8.00 to a swarm mount, a wooden board fixed on top of a 1-meter tall stick [24]. The queen cage was fixed to the upper center of the board, and two feeders were placed on top of the board as a temporary food source for the swarm. We then carefully opened the swarm box and shook all the bees at the base of the swarm mount stand. Within about 1 hour the bees clustered around the queen and began to resemble a natural swarm; scouting activity usually started 0.5-1 hour after the cluster was formed. As soon as the first actively dancing scouts appeared on the swarm performing a clearly direction-coded waggle dance, we caught each scout with a soft forceps before she could recruit and dropped her immediately into liquid nitrogen to preserve brain transcriptional profiles. Within 1 hour, 21-25 scouts were collected and frozen, all during the initial searching phase when the waggle dance of each scout pointed to a different direction. The next morning, a similar number of nest-site recruits were identified as dancing bees during the "consensus" phase [25], the period just prior to the swarm taking off, when all the dances were pointed in the same direction. These recruits were also collected in liquid nitrogen. Both nest-site scouts and recruits were collected in the morning between 9.00-12.00 to eliminate the circadian effect on brain gene expression.

For testing the percentages of nest scouts or recruits turning into food scouts, we applied a combined method of artificial swarming and hive-moving assay (except for one colony that was from a natural swarm, see Methods section in ref. 15). Nest scouts and recruits were labeled as described above, and were given a hive box with five frames as their new home. One of the five frames was pre-filled with honey while the rest were empty. Queens were released to the hive box carefully and the hive entrance was sealed off in the late evening when the swarm bees had settled inside. The procedure used to subsequently collect food scouts and recruits is described in the hive-moving section above.

Brain dissection and RNA extraction

Bee heads were freeze-dried at -80°C, and then whole brains were dissected out in 100% ethanol on dry ice and stored at -80°C [26]. RNA was isolated from whole brains using TRlzol® Reagent (Invitrogen, CA) and RNeasy® Mini Kit (Qiagen Sciences, Maryland). RNA extraction was carried out as per manufacturer's instructions for total RNA and for on-column DNase I treatment (Qiagen). RNA yields and purity were determined with a NanoDrop® ND-1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Delaware).

Microarray experiments and data analyses

We used the honey bee whole genome microarray, which was designed primarily based on the Honey Bee Genome Sequencing Consortium "Official Gene Set" [27] and previously characterized [28]. The microarray has a total of 13,614 probes, and they will be referred to as genes henceforth. A total of 120 bees were used in the experiment. For food scouting, 30 scouts and 30 recruits were sampled from three SDI colonies, with 10 bees per

behavioral group per colony. For nest scouting, 30 scouts and 30 recruits were sampled from two SDI colonies, with 15 bees per behavioral group per colony. The samples were compared in an interconnected loop design with a total of 138 microarrays (Fig 3.3). RNA was extracted from bee brains, and 500 ng per brain were amplified using amino-allyl MessageAmpII (Ambion/Applied Biosystems, Austin, Texas) kits. Of this amplified aRNA, 15 ug were conjugated with Cy3 or Cy5 mono NHS ester (GE Healthcare). From this conjugated aRNA, 3ug of Cy3 and 3ug of Cy5 labeled aRNA were combined and fragmented. The labeled aRNA was loaded on custom-printed Apis microarray slides and hybridized overnight using Agilent coverslips (Agilent, Santa Clara, CA) in a rotating hybridization oven at 42°C. Hybridized arrays were scanned on an Axon 4000B scanner and the images analyzed using GENEPIX 6.1 software (Agilent, California). Images were manually edited for aberrant spots.

Fluorescence intensity spots were filtered if flagged by the scanning software or if the intensity did not surpass the median intensity of the negative control spots for each channel. A Loess transformation was used to adjust the log2 transformed gene expression intensities for dye effects within each microarray, and duplicated spots within the microarray elements were averaged. Gene intensities within each microarray were centralized to allow analysis across microarrays, and microarray elements with more than one missing observation were removed from the analysis.

Statistical analysis of the microarray data was performed with a linear mixed effects model implemented using Restricted Maximum Likelihood (REML) to describe the normalized

log2 transformed gene intensity values, including the effects of dye, behavioral group, bee and microarray. Differences in mRNA abundance were evaluated with a F-test statistic; F1-type False Discovery Rate p-values including multiple-test adjustment was used to generate lists of differentially expressed genes. A total of 11,886 genes remained after the statistical test; 10,001 probes represented genes that were expressed on ≥ 104 microarrays (out of 138 total, 75%). Other probes were excluded from further analysis. Genes that were highly expressed in the hypopharyngeal glands were also excluded due to the risk of contamination during brain dissection [29]. Gene annotation was based on Honey Bee Genome OGS 3.2 (updated, July 2012). Six pair-wise contrasts were tested within ANOVA across four groups, and four pairs were analyzed further: scouts vs. recruits within each context (FS vs. FR and NS vs. NR) and foragers vs. nest hunters within each role (FS vs. NS and FR vs. NR). Two main factors, "role" and "context," and their interaction, " role × context," were tested separately in a Mixed Model ANOVA on the same data. "Context" refers to either searching for food or nest sites, and "Role" refers to the role played in the pursuit of these tasks, either scouting or being recruited. These relationships are illustrated in Fig 3.1A.

Functional Enrichment analysis was performed with DAVID bioinformatics resources 6.7 (http://david.abcc.ncifcrf.gov) [30]. Orthologs of *Drosophila* genes that were significantly differentially expressed were analyzed against a background set of all the *Drosophila* orthologs in honey bee genome Amel 4.5, to identify functionally enriched genes in Gene Ontology (GO) and enriched pathways annotated by the Kyoto Encyclopedia of Genes and Genomes (KEGG Pathways). *Drosophila* orthologs were based on the *Drosophila*

genome Dmel r5.42. Functional enrichments were tested using a hypergeometric tests after correction for multiple testing - Benjamini-Hocherg FDR. Linear Discriminant Analyses were performed using the "lda" function in the MASS package of R software (version 2.15.1). Hierarchal clustering and heatmaps were generated by "pheatmap" package. Class prediction analysis was performed in MeV (version 4.8) using the Supporting Vector Machine (SVM) method [31] and the Uncorrelated Centroid Shrunken (USC) method [32]. Area-propositional Venn diagrams were generated in BioVenn [33]. The relationship map across four behavioral groups in Fig 3.1B was constructed using an "expression distance", where the distance between two behavioral groups was proportional to the number of differential expressed genes in that comparison.

RESULTS

1. *Differences in brain gene expression across four behavioral groups.*

There were extensive differences in brain gene expression between food scouts and recruits and between nest scouts and recruits. Over 1000 genes were differentially expressed between field-collected scouts and recruits in each context (Table 3.1, FDR < 0.05 with contrast $p < 0.005$). These genes made up approximately 10% of the total genes analyzed (1032 out of 9877 and 1003 out of 9889, respectively), which was very similar to the number of gene expression changes in our previous study of food scouts in a semi-natural enclosure [15]. There also were extensive differences in the brain gene expression profiles between food scouts and nest scouts, as well as between food recruits and nest recruits (Table 3.1). There were multiple causes of these differences, including differences in social environment, tasks performed, and searching experience. In addition, there were similar

numbers of genes differentially expressed between nest scouts and food recruits as between scouts and recruits from the same context (Table 3.1). However, this was not true for the comparison between food scouts and nest recruits, where 2 times more genes were differentially expressed (Table 3.1).

We used these results to generate a testable prediction based on the hypothesis that behavioral plasticity would be limited when dramatic transcriptional changes are required to switch between behavioral states [34]. In other word, the "closeness" between the two behavioral states in term of brain gene expression may be positively correlated with one behavioral state's "likelihood" to change into another state. To accomplish this, we used our data (Table 3.1) to construct a relationship map across four behavioral states - two types of scouts and recruits - based on this hypothesis. A "relationship distance" that was proportional to the number of differential expressed genes in each comparison was used (Fig 3.1B). This relationship map confirmed that the magnitudes of gene expression changes were similar between scouts and recruits across contexts. It also predicted an interesting asymmetry: food recruits are equally likely to become food or nest scouts, but nest scouts are more likely to become food scouts than are nest recruits.

We further tested the latter prediction in a behavioral study. Among the 5 colonies tested, the nest scout data were published [15] and the nest recruit data were not. Our behavioral tests (Fig 3.4) confirmed this prediction in the newly settled colonies observed in early summer (June, the same month as the bees used in the microarray were collected). Nest recruits were ≥ 3 times less likely to become food scouts (average 9% across 3 colonies)

than nest scouts were (average 31% across three colonies). Notably, this difference disappeared in the two colonies tested in July, a late season for swarming, most likely due to urgent colony foraging need [21].

2. *Similarities in brain gene expression of scouts across distinct contexts.*

Despite their many differences in behavior and the noted differences above, there were strong similarities in the brain gene expression profiles of nest and food scouts (Table 3.2). There was a significant overlap between the two scouting gene lists (FS vs. FR list, and NS vs. NR list), consisting of about 1/3 of the genes in each list (344 genes, RF= 3.3, $p<0.0001$). Expression patterns for food and nest scouts were also significantly correlated (Pearson's correlation, $R^2=0.31$, $p<0.00001$), with 81% showing differences relative to recruits that were in the same direction. We also found that the enriched GO terms (Fisher's exact test, EASE score, $p<0.05$) shared between nest and food scouts significantly overlapped (10 terms out of 45 and 36, RF=18.6, $p<0.0001$).

Gene Ontology enrichment analysis also showed there were distinct sets of functional enrichments for food scouts and nest scouts (Table 3.4). The highly enriched GO terms in food scout brains (Hypergeometric test with Benjamini FDR <0.05) were biological processes involved in up-regulation of glucose metabolism process and down-regulation of protein folding. Enrichments for the KEGG pathways include an up-regulated glycolysis/ gluconeogenesis pathway and an up-regulated citrate cycle (TCA) pathway. For nest scouts, we found a suite of highly enriched GO terms related to biosynthesis and structure of ribosomes and cellular compartment "lipid particles." Highly enriched KEGG pathways

included an up-regulated ribosome pathway and two down-regulated pathways related to degradation of branched-chain amino acids (valine, leucine, and isoleucine) and metabolism of butanoate. Food and nest scouting behaviors shared the GO cellular compartment term "lipid particle" in up-regulated genes, and the GO molecular function term "protein folding" for down-regulated genes (Hypergeometric test with Benjamini FDR <0.05).

Two transcription factor genes, *forkhead box P* (*foxP*, or *fd85E*) and *Ftz-transcription factor 1* (*ftz-f1*) showed consistent up-regulation in both food scout and nest scout brains. Another transcriptional factor gene, *broad-complex* (*br-c*, or *br*) was up-regulated in the nest scout brains. These three transcription factor genes had previously been identified as "master-regulators" that potentially orchestrate important behavioral and maturation-related gene expression networks in the honey bee brains, including hormonal signaling [35]. Moreover, nest scout brains showed significantly higher expression for a suite of hormonal signaling genes, such as *ultraspiracle* (*usp*), *ecdysone-induced protein 75*, *ecdysone-regulated gene E74*, *hormone receptor-like 38*, *dopamine/ecdysteroid receptor(dopecr)* (Table 3.6A) and were enriched for GO terms "response to ecdysone" and "response to steroid hormone stimulus" (EASE score<0.05). Some of these genes such as *hormone receptor-like 38* were also significantly differentially expressed between food scouts and recruits but were down-regulated in food scout brains. Ecdysone receptor gene *ecr* was also down-regulated in food scout brains (Table 3.6B). These results suggest an intriguing relationship between endocrine processes and the development of both scouting behaviors.

We found strong similarity between the neurogenomic profiles of food scouts in this study and those of food scouts in our previous study [15](RF= 1.6, $p<0.0001$, Table 3.2), where a different experimental environment and different behavioral assays were used. There was also a significant overlap between brain gene expression profiles in the comparison between nest scouts and the previous food scouts (RF=1.8, $p<0.0001$, Table 3.2). The transcription factor genes *foxP* and *ftz-f1* showed a robust up-regulation pattern across three scouting experiments, and they were among 84 genes showing this pattern. Among the 10 enriched GO terms that were shared between food and nest scouts, 8 of them were also shared with the food scouts collected in Liang et al. [15].

We had previously reported [15] that monoamine, glutamate and GABA-related neural signaling genes were involved in regulating scouting behavior. In the present study, we found the same neurotransmitter systems were associated with both food and nest scouting behaviors. In addition, we found that four acetylcholine-related genes, encoding three enzymes and one vehicle transporter, also showed significant differential expression in scouts compared to non-scouts. We assembled a set of 16 genes that encode receptors, transporters, and enzymes in monoamine, glutamate, GABA and acetylcholine systems, and found that 8 and 9 genes were differentially expressed in food scouts and nest scouts, respectively, compared to recruit bees in the same context (Fig 3.2A). Among them, dopamine receptor 1 gene (*dopr1)*, iontropic glutamate receptor 1 (*glu-r1)*, metabotropic GABA-B receptor subtype 3 (*mgarb3),* and excitatory amino acid transporter 4 (*eaat-4)* were significantly associated with both food and nest scouting. Linear Discriminant Analysis using 16 neural signaling genes clearly separated 30 individual food scouts and

30 food recruits based on their expression patterns (LD1 and LD2 accounted for 75.2 % of the variation, Fig 3.2B). This analysis also distinguished 30 individual nest scouts and 30 nest recruits (LD1 and LD2 accounted for 90.5% of the variation) (Fig 3.2C). When food and nest scouting individuals were combined, these 16 signaling genes clearly distinguished the bees either by behavior or by context (LD1 and LD2 accounted for 85.0% of the variation, Fig 3.5A).

By contrast, genes involved in serotonin and tyramine signaling did not differentiate scouts from non-scouts. There was no gene involved in serotonin or tyramine signaling that showed differential expression between scouts and recruits in food or nest contexts. We used five genes in serotonin and tyramine signaling for Linear Discriminant Analysis, and they did not distinguish scouts within each context or when both contexts were combined (Fig 3.2 D-E and Fig 3.5B).

3. *Differences in brain expression of scouts across distinct contexts.*

The expression differences between the two types of scouts are more than 1.5 times larger than the similarities between them (Table 3.1 & Fig 3.1B). Similar expression differences were observed between the two types of recruits. Interestingly, these two neurogenomic profiles showed a significant overlap and a high level of concordance. An average of 45% of the differentially expressed genes overlapped between the FS vs. NS list and the FR vs. NR list, and 92% of them changed in the same direction. We found no functional GO term highly enriched for the differentially expressed genes between nest and food scouts, nor between nest and food recruits (Benjamini FDR <0.05). There were also much fewer GO

terms (EASE score $p<0.05$) that overlapped between the FR vs. NR list and the FS vs. NS list (only 2 overlapping terms out of 41 and 26 terms respectively). However, functional enrichment analysis based on the protein database SWISS-PROT revealed that these two profiles were both enriched for genes encoding proteins involed in "alternative splicing" and "calmodulin-binding" (SP_PIR_Keywords, EASE score $p<0.05$), which may suggest different functional roles for these biological processes in foraging versus in a nest-hunting context.

4. ***Factorial analysis of brain gene expression patterns associated with "role" and "context" differences.***

To integrate the pair-wise comparison results described above, we performed additional analyses on the brain gene expression differences associated with "role" and "context." The "role" difference represents the contrast of scouts versus recruits, the different roles played in searching behaviors, and the "context" difference represents the contrast of nest hunters and foragers, two very different ecological contexts in which the scouting behavior took place (Fig 3.1A). Our results showed that approximately the same numbers of neurogenomic changes, over 1000 genes, were associated with differences in "role" (FDR<0.05 with contrast $p<0.005$, Table 3.1, Fig 3.1C). Gene Ontology analysis confirmed that the category cellular component "lipid particle" was highly enriched for differences in "role" (Benjamini FDR $p<0.05$). This result suggests that differences in lipid transportation and metabolism in the brain are present between scouts and recruits in both behavioral contexts. GO terms such as "response to ecdysone" and "response to steroid hormones" were also among the enriched terms for "role" (EASE score <0.05), but

because most of these genes were expressed in different patterns across contexts, only the gene encoding juvenile hormone acid methyltransferase was significant for "role" without interaction with the "context" (Table 3.6C).

We found that over 2000 genes, 1.8 times more than those associated with differences in "role," were associated with differences in "context" (FDR<0.05 with contrast p<0.005), Table 3.1, Fig 3.1C), but they showed no predominant molecular pathway or GO functional enrichment underlying this difference (Benjamini FDR p>0.05). These genes contained fewer functional enrichments (EASE score p<0.05) than those associated with "role" did (Fig 3.1D), in spite of exhibiting a much larger neurogenomic response. Among the significant functional categories identified using a lower stringency, "response to ecdysone" and "response to steroid hormone stimulus" were significantly associated with "context" (EASE score p<0.05). The KEGG pathway "glycolysis/gluconeogensis" was also significantly associated because the differentially expressed genes in this pathway were only present in forgers. NMDA-type iontropic glutamate receptor gene *nmdar1* (GB46886) was not significantly associated with either type of scouting behavior, but was identified as significantly associated (FDR<0.05, contrast p<0.005) with "context" and was more highly expressed in a nest-hunting context than in a foraging one.

A relatively large set of genes, 864 of them, showed a significant interaction between "context" and "role." Their expressions in scouts differed based on the context (Table 3.1). One of these genes, honey bee odorant binding protein 4 (*obp4* or *asp4*) was singled out as the most up-regulated gene in nest scouts compared to nest recruits (FDR<0.05, contrast

$p<10^{-20}$), but was only modestly down-regulated in food scouts in this study (FDR<0.05, contrast p =0.012) (Fig 3.6A). *Obp4* gene has been associated with a variety of foraging activities in previous microarray studies (Fig 3.6B). Several neurotransmitter receptors also showed varied expression patterns and an interaction between role and context (see Fig 3.1A).

5. ***Molecular signatures of scouting across distinct behavioral contexts***

Based on the factorial analysis mentioned above, we selected a set of 557 genes that were only associated with the role of scouts and independent of context and the interaction between role and context. We further bioinformatically identified a minimum list of 89 genes as the "best predictors" for scouting behavior (Fig 3.7, gene list: Table 3.5), and successfully predicted a bee's role as a scout or a recruit 92.5% of the time ("leave-one-out" cross-validation), Specifically, 111 out of 120 bees were identified correctly as a scout or recruit (54 out 60 bees correct for scouts, 57 out of 60 bees correct for recruits), compared with 78.3% success if all 557 genes were used without any selection process (Table 3.3A).

Among the 89 best predictor genes, the gene encoded the transcription factor protein Forkhead box P (*foxP)* was consistently up-regulated in the brains of food scouts in the field or in the controlled environment and also in the brains of nest scouts. Similarly, the gene encoded a small heat-shock protein Lethal essential for life (*l(2)efl-like*, GB45906) showed a consistent down-regulation pattern in scout brains across all three scouting experiments. *Metabotropic GABA receptor type B 3* (*mgarb3*) gene was up-regulated in

food and nest scouts brains in this microarray study, which continues to support the involvement of GABA-related genes as one of the "best predictors" for scouting behavior (GABA transporter gene *gat-a* was one the "best predictors" for food scouts [15]).

Could identifier genes for nest scouts correctly predict individual bees as food scouts and *vice versa*? Using the same class prediction methods, our results showed that the best identifier genes for nest scouting indeed predicted a bee's role in food scouting significantly better than random (39 out of 60, 65% success, binomial test, $p<0.05$), but not *vice versa* (31 out of 60, 58% success, binomial test, $p>0.05$) (Table 3.3B). This analysis suggested that either nest recruits were better identified than food recruits, or the molecular signature of nest scouting behavior is a more stringent representation for scouting behavior than that of food scouting.

DISCUSSION

The principal significance of these results is that they support scouting in honey bees as a behavioral syndrome or personality trait. Despite the differences in behavior and context, there are common patterns of brain gene expression for both types of scouts. There are also substantial differences in brain gene expression between the two types of scouts, but due to the differences in the way the behaviors are performed and the contexts in which they occur we did not expect identical patterns of brain gene expression. Importantly, our results suggest there is a common core of genes that in some way is associated with the tendency to act like a scout and seek out novel resources rather than wait to be recruited to

them. These results are highly consistent with the findings of Liang et al. [15], which suggests that scouting in honey bees is a manifestation of personality because of its behavioral consistency across foraging and nest hunting contexts and the commonalities in underlying molecular pathways with those of novelty-seeking in humans.

These data are also helpful for addressing the evolutionary question of why personality, which limits plasticity in behavior, occurs despite potential fitness costs. Our findings of similar brain expression patterns in several key neural signaling genes provides molecular snapshots of a common neural mechanism for scouting behavior across contexts, suggesting that the limited behavioral plasticity in food and nest scout bees may derive from shared mechanisms that both behaviors rely upon. Our results extend beyond the previous findings of glutamate and catecholamine as the molecular determinants of scouting behavior [15], indicating that the molecular bases of scouting are not limited to food searching but underlie a suite of behavioral traits related to how individuals explore their environment. In addition, this limited plasticity in scouting tendency was robust not only across ecological contexts, but persists in varied environmental conditions (semi-natural enclosure or field) and in different experimental methods (two different food scouting assays used).

Beyond the shared molecular signatures of the two scouting behaviors, there were also large changes in brain gene expression associated with behavioral context. One reason for these expression differences lies in the different social environments. Food scouting occurs daily in normal colonies, while nest scouting occurs only 1-3 times a year during colony

reproduction, or swarming, in which one colony splits into two and produces a homeless colony (swarms) that must select a new nest. Expression differences between contexts may also reflect the presence or absence of waggle dances during scouting due to a difference in collection methods. Food scouts were collected before they could ever recruit other bees, so dance performance was absent around these food scouts. Nest scouts could only be identified while they perform waggle dances. Therefore, they were collected shortly after waggle runs so they may have been at a higher level of excitement than food scouts were. Other differences include task-specific differences, such as the distinct search images and potential variation in flight distances between the two types of scouts. Food scouts search for colorful flowers while nest scouts search for dark tree holes, and it usually takes longer to find a proper tree hole (30-60 min) than a proper flower patch (15-20 min). Interestingly, despite those differences, the overlapping expression profiles between two types of scouts and two types of recruits were highly concordant, which suggest that scouts and recruits have similar genomic responses to these different contexts.

Our discovery of substantial transcriptomic differences between nest scouts and nest recruits challenges the traditional notion of nest scouts as being all the bees actively participated in nest selection process, and as a result, provided new insights for inferring individual differences in this behavior. A nest scout (also called nest-site scout) is traditionally defined as any worker bee that actively participates in the nest-site selection process during swarming [19, 20, 25]. Since the only distinction in our study between a nest scout and a nest recruit is who initiates the search, we confirmed that the molecular signature for scouting behavior indeed captures the novelty-seeking nature of scouting

behaviors. The significant overlap in neurogenomic profiles between nest scouts and food scouts fortifies the conclusion that individual differences in novelty seeking are a common characteristic of scout bees in both nest hunting and foraging. Moreover, the molecular signature of nest-scouting behavior was suggested by class-prediction analysis to be more powerful for predicting scouts across contexts than was food-scouting behavior. The significant difference between nest recruits and nest scouts in their likelihood to become food scouts also supports an important distinction between the initial, independent nest-hunting bees (nest scouts) and their followers that later visit potential nest sites and dance (nest recruits). This challenges the traditional grouping of all bees that dance for potential nest-sites together as nest-site scouts [18]. This result also suggests that using brain transcriptomic approach to analyze correlated behaviors could offer us new understandings of behavioral syndromes.

Some genes respond to context changes by their expressions in the brain [36, 37]. The *odor-binding protein 4* (*obp4*) gene, one of the most up-regulated genes in nest scout brains, was expressed in the opposite direction in food scouts in this study. Interestingly, it was the most down-regulated gene in food scout brains studied in the controlled environment [15], and was down-regulated in the brains of active foragers compared to inactive ones in both the morning and afternoon [36]. These results suggest that the *obp4* gene may respond to different types of olfactory cues associated with food or nest "search images" through its expressional changes.

Scouting is a fascinating behavior that is performed by only a fraction of worker bees. We made some progress in elucidating its molecular basis, but how this behavior develops remains unknown. Previous research showed that both nest and food scouting tendencies have a heritable component; certain genotypes are overrepresented among scouts, and a colony's scouting rate is affected by patriline diversity [20, 22, 38]. Are these predispositions toward scouting established at an early developmental stage? Does nutrition or hormonal regulation play a role? Diet and endocrine function influence behavioral maturation in the dynamic transition from nurse bees to foragers [39,40], but their effects have not been studied for same-age bees such as scouts and recruits. In this study, we found that brain gene expression changes were associated with endocrine processes in both types of scouting behaviors but apparently function in different ways. It is possible that different diets or social environments may interact with heritable elements in individual bees and regulate endocrine systems during the early developmental stage to affect their scouting tendency.

Animal personality leads to limited plasticity in behavioral responses and is thought to impose fitness costs on individuals [16], but social interactions among honey bees allow personalities to enhance colony fitness. This discrepancy can be explained by eusociality. In insect societies, selection acts at the level of the colony [41] to favor those that can precisely regulate internal and external conditions. The collaboration between scouts and recruits increases foraging efficiency by allowing the colony to both monitor the fluctuation of floral availability and nectar flow in the environment and devote most foragers to exploiting the best patches while they are blooming [21, 42]. Thus, these

behavioral types are essential for maintaining constant food influx despite competition from other pollinators. This social foraging is more important during the early and late parts of the foraging season when floral resources are sparse but less so when forage is plentiful [42]. Our studies on the molecular bases of scouting behavior suggest that, by different "tuning" of an individual's neural system, such personality can be produced and regulated, to enhance overall fitness of the group.

Division of labor for tasks such as thermoregulation, undertaking, and guarding are also based on behavioral tendencies with strong genetic components [20, 22, 38, 43], but it is not clear if these tendencies remain consistent over time or different ecological contexts. In thermoregulation behavior, genetic diversity in the hive produces different response thresholds to temperature changes among hive bees; each individual only responds to a limited temperature range that is slightly different from other bees, preventing excessive response to temperature fluctuation [43]. This limited individual plasticity in response to temperature change collectively enhances colony efficiency in a social context by preventing an excessive colony-level response. It will be interesting to study if thermoregulation behavior or any other types of division of labor in honey bee colonies also exhibit personality, showing consistency across time and contexts.

To summarize, through comparative brain transcriptomic analyses, we confirmed the shared molecular signatures for scouting behaviors across distinct ecological contexts, foraging and nest hunting, suggested that a common core of genes underlie both scouting behaviors. These findings further supported the idea of scouting tendency as an animal

personality and begin to dissect the mechanistic basis of limited plasticity in behavior. They also contribute to the study the evolution of personality, particularly within animal societies.

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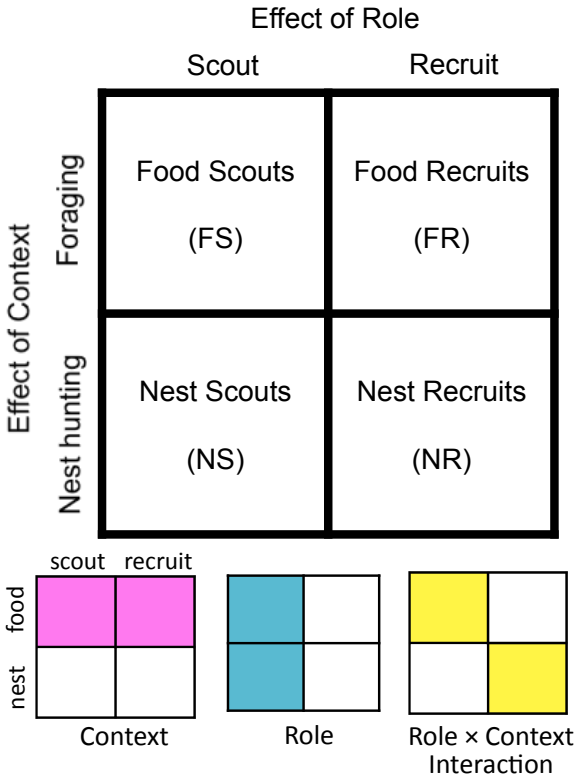
FIGURES AND TABLES

Figure 3.1 Experimental design and result outlines for the four behavioral groups and two main factors analyzed in this study. **(A)** Experimental design and the four behavioral groups analyzed in this study. Effect of role was analyzed by comparing scouts with recruits, and effect of context was analyzed by comparing foragers and nest hunters. **(B)** A relationship map among the four behavioral groups analyzed was drawn in proportion to the numbers of significantly differentially expressed genes (DEG) between each group. Green lines show that similar numbers of genes had expressed changes among FS-FR, NS-FR and NS-NR comparison. Blue line show the biggest distance among six pairs was FS-NR comparison. **(C)** Area-proportional Venn diagram showing the numbers of differentially expressed genes (DEG) and their overlap among the effect of role, the effect of context, and their interaction. **(D)** Area-proportional Venn diagram showing the number of functional enrichments (enriched Gene Ontology terms and KEGG pathways) and their overlap among the effect of role, the effect of context, and their interaction. Notice that the effect of context and the role-by-context interaction have a reversed relationship between their numbers of DEGs and the functional enrichments of these DEGs.

A.



B.

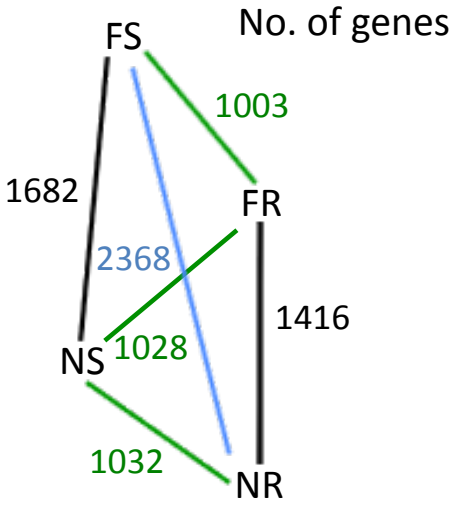
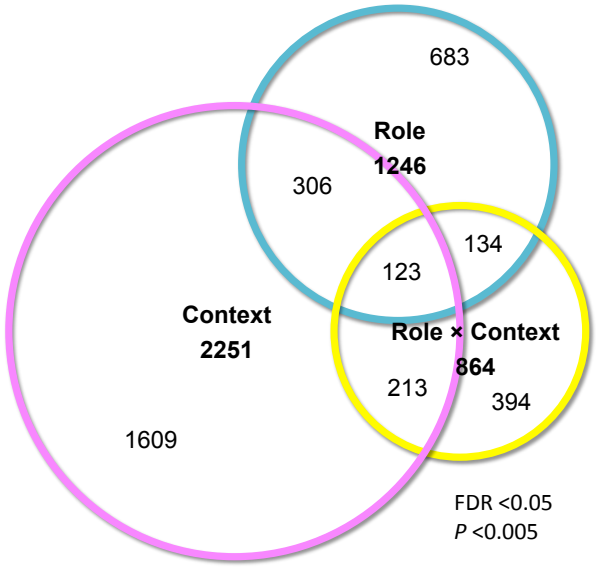


Figure 3.1 (cont.)

C.



D.

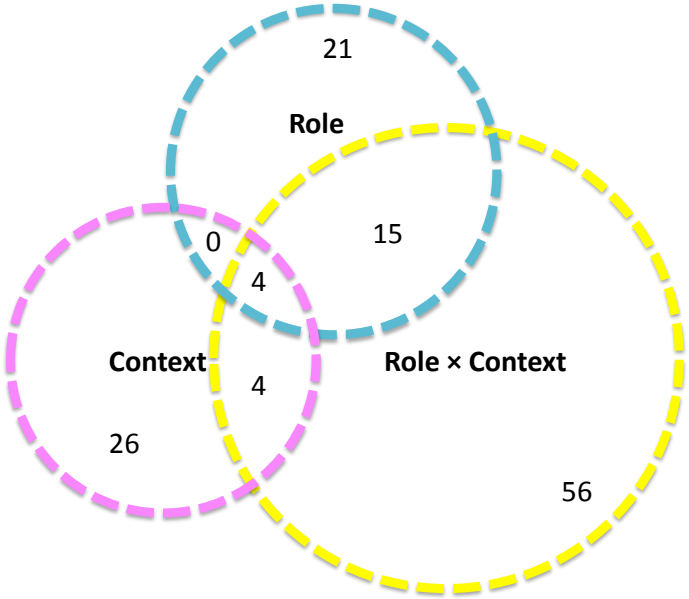


Figure 3.2 Expression analyses of 16 neurotransmitter genes in food scouting and nest scouting. **(A)** Expression ratio of 16 neurotransmitters genes in food scouting and nest scouting. Yellow bar: expression ratios of nest scouts divided by nest recruits (NS/NR); Orange bar: expression ratios of food scouts divided by food recruits (FS/FR). * FDR<0.05, contrast p<0.005. **(B-C)** Linear Discriminant Analysis (LDA) shows a clear separation of scouts and recruits in 60 individual bees, plotted by food scouting (LD1 and LD2 accounted for 75.2 % of the variation) and nest scouting(LD1 and LD2 accounted for 90.5% of the variation), respectively, using the expression profiles of 16 neurotransmitter system genes in dopamine, octopamine, glutamate, GABA and acetylcholine systems. FS: food scouts (red), FR: food recruits (blue), NS: nest scouts, NR: nest recruits, with different colonies denoted as subscript 1, 2 and 3. **(D-E)** LDA shows no separation of scouts and recruits in the same 60 individual bees, plotted by food or nest scouting, using the 5 genes in the serotonin and tyramine systems. Individual bees were labeled the same as in (B-C).

A.

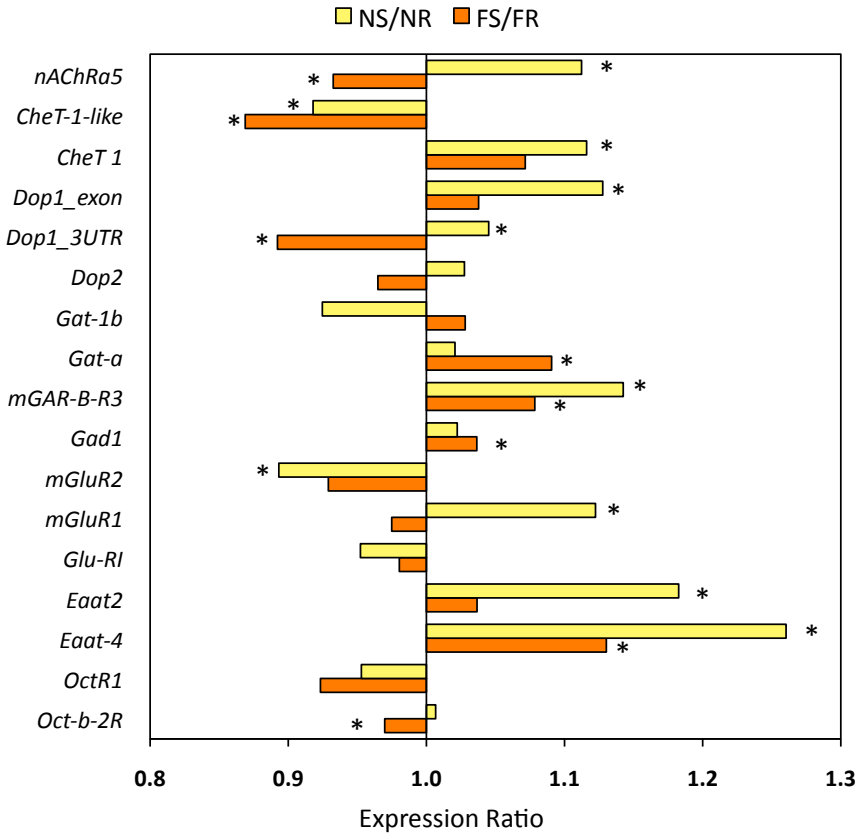


Figure 3.2 (cont.)

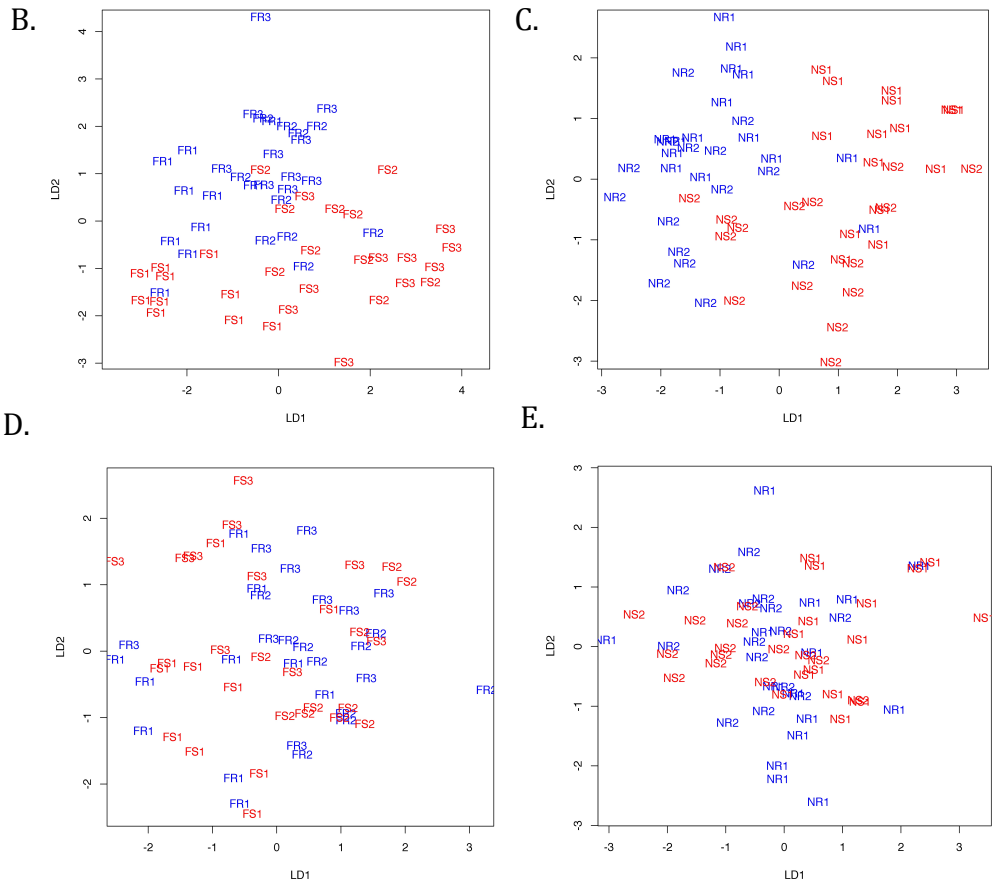


Figure 3.3 Microarray loop design for the comparative brain gene expression between two scouting behaviors. FS: food scouts, FR: food recruits, NS: nest scouts, NR: nest recruits. B1-12, B1-13 and B1-14 were three colonies used to collected food scouts and recruits. B1-12 and B2-17 were two colonies used to collected nest scouts and recruits. A total of 138 arrays were used. Detailed description of sample use was in the Material and Methods section of the main text.

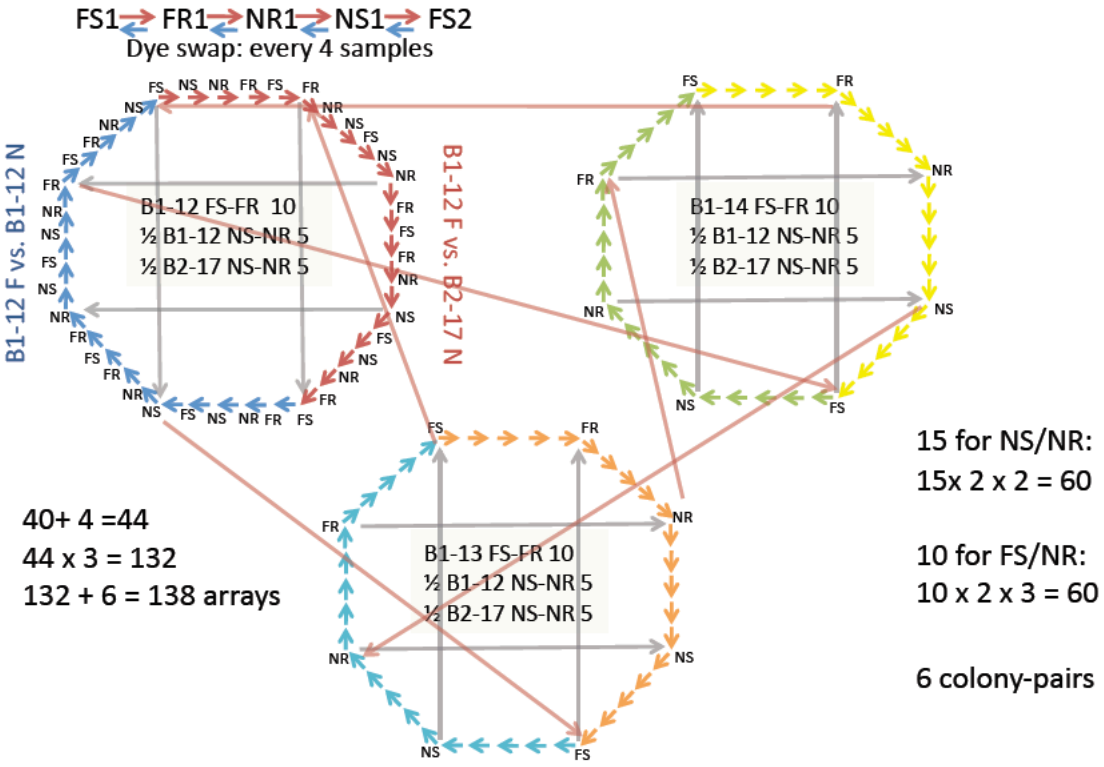


Figure 3.4 Behavioral test results comparing the probabilities of two types of nest hunters turning into food scouts after the swarm found a nest and started regular foraging activity. Blue bar: best scouts. Light yellow bar: nest recruits. AS: artificial swarm, NS: natural-occurred swarm. Fisher's exact test: * $p<0.05$.

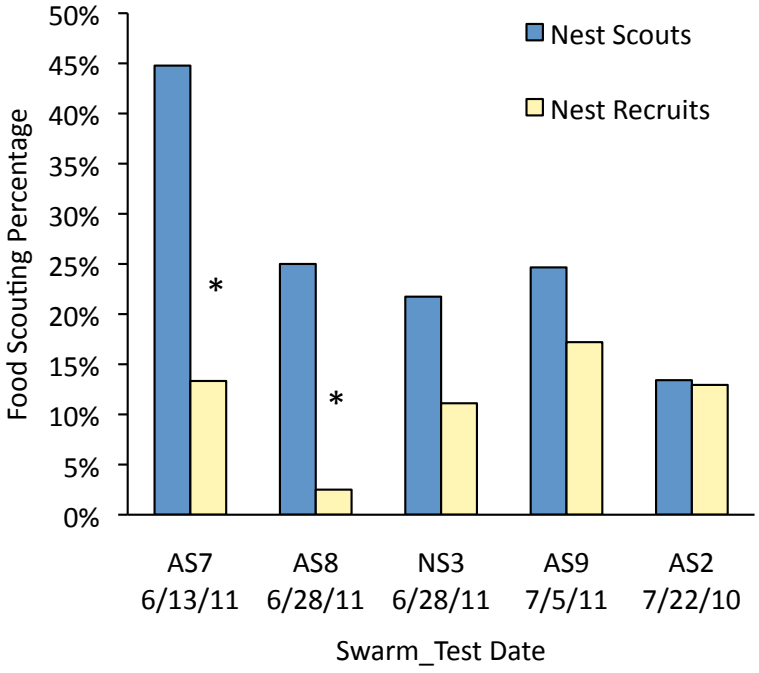


Figure 3.5 Linear Discriminant Analysis (LDA) results using neural signaling genes when the four behavioral groups were combined. **(A)** Using 16 neural signaling genes in dopamine, octopamine, glutamate, GABA, and acetylcholine systems, LDA clearly distinguished the 60 individual bees either by behavior or by context (LD1 and LD2 accounted for 85.0% of the variation). **(B)** LDA failed to separate the four groups either by context or by role when using 5 neural signaling genes in the serotonin and tyrimine systems.

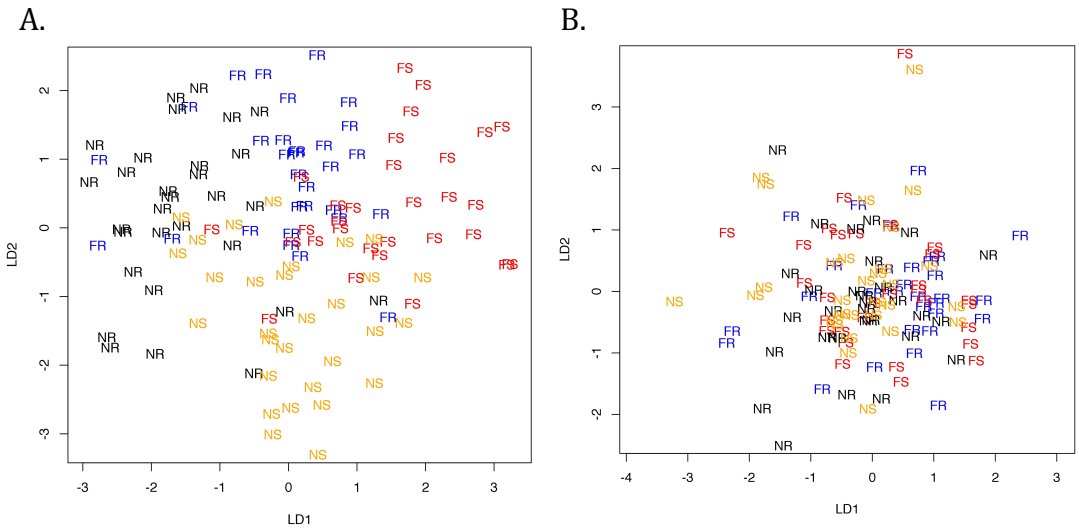


Figure 3.6 Log 2 brain expression values of *Odor binding protein 4 (obp4*) gene **(A)** among the four behavioral groups during scouting, and **(B)** across different behaviors. In (A), the *obp4* expression patterns shows an up-regulation pattern and the highest fold change between nest scouts and recruits and a moderate down-regulation in food scouts in comparison to food recruits (both experimented in the field environment, FDR<0.05, contrast $p<0.05$). In (B), the *obp4* gene was up-regulated in food scouts compared to non-scouts (experiments in semi-natural enclosure), and in foragers compared to nurses. This gene was down-regulated in active foragers compared to inactive foragers during the morning (AM) and afternoon (PM), all at FDR<0.05, contrast $p<0.005$ level. *Obp4* was also found to be significantly down-regulated (at FDR<0.05 without contrast test) in high pollen-hoarding foragers, but the fold change was very marginal.

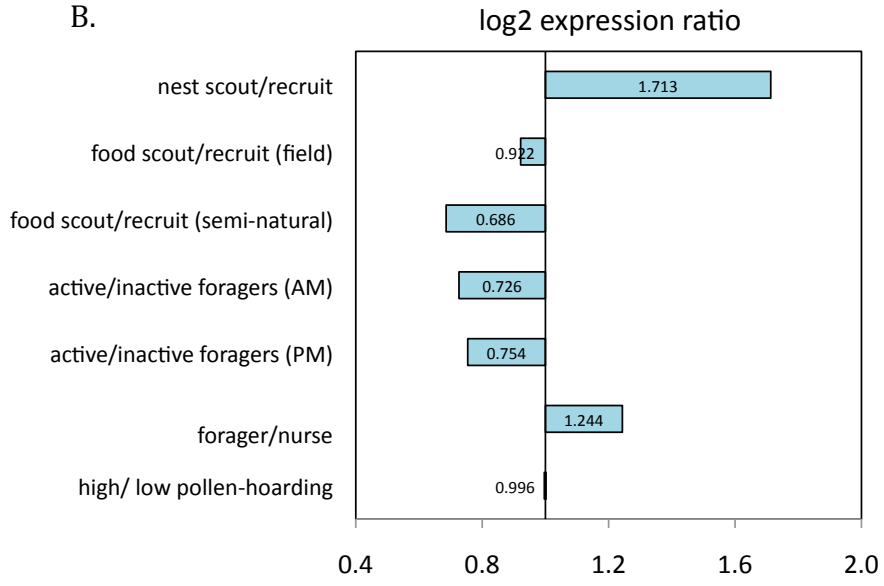


Figure 3.7 Hierarchical Clustering result was shown in heatmap for the 89 best predictor genes across the expression values of four behavioral groups (group mean). FS: food scouts, FR: food recruits, NS: nest scouts, NR: nest recruits.

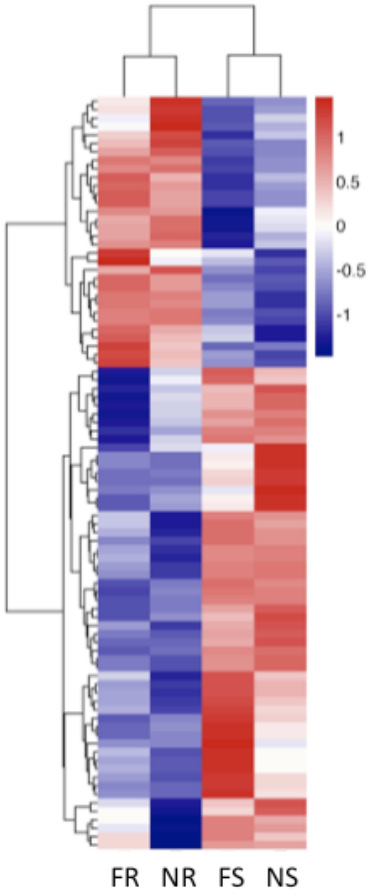


Table 3.1 Numbers of significantly differentially expressed genes in each analysis (FDR<0.05, contrast p<0.001, except for "interact", which is FDR<0.05, no contrast available). FS=food scouts, FR=food recruits, NS=nest scouts, NR=nest recruits. *Interact: the interaction between Context and Role (Context x Role).

FS vs. FR	NS vs. NR	FR vs. NR	FS vs. NS	FS vs. NR	NS vs. FR	Context	Role	Interact
459	522	778	895	1206	441	1215	585	
541	510	638	787	1162	587	1036	661	
1003	1032	1416	1682	2368	1028	2251	1246	864*

Table 3.2 Scouting-related brain gene expression across contexts: number of differentially expressed genes and enriched Gen Ontology categories overlapping in three experiments. Data are from…N=9877 (total gene); N=5195 (total GO) Expected =n*D/N. Food[1]: food scout vs. recruits collected in the field experiments. Food[2]: food scouts vs. non-scouts collected in the semi-natural enclosure.

Experiments	Genes				Gene Ontology Categories			
	Expected	Observed	RF	P value	Expected	Observed	RF	P value
Food[1] × Nest	104.8	344	3.3	< 0.0001	0.54	10	18.6	< 0.0001
Food[1]× Food[2]	123.8	201	1.6	< 0.0001	0.76	8	10.5	< 0.0001
Nest × Food[2]	127.4	230	1.8	< 0.0001	1.05	26	24.8	< 0.0001
Food[1]× Nest × Food[2]	---	84	---	---	---	8	---	---

Table 3.3 Class prediction results using uncorrelated shrunken centroid (USC) method. (A) Best predictor genes selected from 557 "role-only" genes. Delta and Rho are user-selected parameters. Gene lists from delta=0.5 are subsets of delta=0, and within each delta value, the selected gene number increases while smaller gene lists are the subsets of the larger gene lists. (B) Cross-context class prediction results, testing how well the identifier genes best for predicting food scouts and recruits predict nest scouts and recruits and *vice versa*. USC: delta=0, Rho=0 was used for both tests in the table. *P* value in red denotes significance ($p<0.005$).

A.

USC: Delta=0	**Rho=0.5**	**Rho=0.6**	**Rho=0.7**	**Rho=0.8**	**Rho=1**	**No selection**
# of predictor genes	89	167	302	428	509	557
# of correct prediction	111	110	110	109	108	94
% of correct prediction	92.5 %	91.7%	91.7%	90.8%	90.0%	78.3%
USC: Delta=0.5	**Rho=0.5**	**Rho=0.6**	**Rho=0.7**	**Rho=0.8**	**Rho=1**	
# of predictor genes	81	145	247	325	371	
# of correct prediction	110	111	111	108	106	
% of correct prediction	91.7%	92.5%	92.5%	90.0%	88.3%	

B.

Significant gene set	**Training individual profiles**	**No. of best identifier genes** (% of correct prediction)	**Testing individual profiles**	**No. of correct prediction**	**Binomial test *p* value**
Food scouting (1003 genes)	30 food scouts & 30 recruits	95 genes (95%)	30 nest scouts & 30 recruits	31/60 bees (58%)	0.449
Nest scouting (1032 genes)	30 nest scouts & 30 recruits	108 genes (95%)	30 food scouts & 30 recruits	39/60 bees (65%)	0.0134

Table 3.4 Functional enrichment analysis results are shown by up- and down-regulated genes in nest scouts and food scouts, respectively. Only the highly significant terms and pathways are shown here (Hypogeometric test with Benjamini FDR<0.05), with the KEGG pathways in blue.

Category	Term	Count	P-value	Benjamini
UP-regulated genes in Nest Scouts				
GOTERM_CC_FAT	GO:0022626~cytosolic ribosome	23	1.41E-16	1.84E-14
GOTERM_CC_FAT	GO:0044445~cytosolic part	25	1.49E-14	1.23E-12
GOTERM_CC_FAT	GO:0022625~cytosolic large ribosomal subunit	17	2.45E-13	1.36E-11
GOTERM_CC_FAT	GO:0033279~ribosomal subunit	25	1.02E-10	4.24E-09
GOTERM_CC_FAT	GO:0005840~ribosome	25	7.84E-10	2.60E-08
GOTERM_CC_FAT	GO:0015934~large ribosomal subunit	19	1.85E-09	5.12E-08
GOTERM_CC_FAT	GO:0005829~cytosol	28	5.62E-09	1.33E-07
GOTERM_MF_FAT	GO:0003735~structural constituent of ribosome	25	3.52E-08	1.48E-05
GOTERM_BP_FAT	GO:0051231~spindle elongation	15	2.96E-07	3.16E-04
GOTERM_BP_FAT	GO:0000022~mitotic spindle elongation	15	2.96E-07	3.16E-04
GOTERM_CC_FAT	GO:0030529~ribonucleoprotein complex	26	1.10E-04	2.28E-03
GOTERM_MF_FAT	GO:0005198~structural molecule activity	28	1.30E-05	2.74E-03
GOTERM_BP_FAT	GO:0035071~salivary gland cell autophagic cell death	11	1.46E-05	7.78E-03
GOTERM_BP_FAT	GO:0035070~salivary gland histolysis	11	1.46E-05	7.78E-03
GOTERM_BP_FAT	GO:0048102~autophagic cell death	11	1.46E-05	7.78E-03
GOTERM_BP_FAT	GO:0007559~histolysis	11	2.55E-05	9.06E-03
GOTERM_BP_FAT	GO:0016271~tissue death	11	2.55E-05	9.06E-03
GOTERM_CC_FAT	GO:0005811~lipid particle	19	5.60E-04	1.03E-02
GOTERM_BP_FAT	GO:0006412~translation	27	5.45E-05	1.45E-02
GOTERM_CC_FAT	GO:0022627~cytosolic small ribosomal subunit	6	1.64E-03	2.68E-02
GOTERM_BP_FAT	GO:0022612~gland morphogenesis	12	1.54E-04	3.25E-02
GOTERM_BP_FAT	GO:0007435~salivary gland morphogenesis	12	1.54E-04	3.25E-02
KEGG_PATHWAY	dme03010:Ribosome	23	1.91E-16	1.18E-14
DOWN-regulated genes in Nest Scouts				
GOTERM_CC_FAT	GO:0005811~lipid particle	25	7.02E-06	1.46E-03
GOTERM_BP_FAT	GO:0055114~oxidation reduction	33	8.38E-06	8.36E-03
KEGG_PATHWAY	dme00280:Valine, leucine and isoleucine degradation	9	7.75E-05	5.10E-03
KEGG_PATHWAY	dme00650:Butanoate metabolism	7	5.20E-04	1.70E-02
UP-regulated genes in Food Scouts				
GOTERM_BP_FAT	GO:0046164~alcohol catabolic process	9	8.93E-06	9.09E-03
GOTERM_BP_FAT	GO:0044275~cellular carbohydrate catabolic process	9	8.93E-06	9.09E-03
GOTERM_BP_FAT	GO:0019320~hexose catabolic process	8	2.01E-05	1.02E-02
GOTERM_BP_FAT	GO:0006007~glucose catabolic process	8	2.01E-05	1.02E-02
GOTERM_BP_FAT	GO:0046365~monosaccharide catabolic process	8	3.04E-05	1.03E-02
GOTERM_BP_FAT	GO:0006006~glucose metabolic process	9	1.13E-04	2.86E-02
GOTERM_BP_FAT	GO:0016052~carbohydrate catabolic process	9	1.86E-04	3.12E-02
GOTERM_BP_FAT	GO:0006096~glycolysis	6	2.30E-04	3.31E-02

Table 3.4 (cont.)

GOTERM_BP_FAT	GO:0051186~cofactor metabolic process	13	1.80E-04	3.61E-02
KEGG_PATHWAY	dme00010:Glycolysis / Gluconeogenesis	10	4.03E-06	2.66E-04
KEGG_PATHWAY	dme00020:Citrate cycle (TCA cycle)	7	7.74E-04	2.52E-02
DOWN-regulated genes in Food Scouts				
GOTERM_BP_FAT	GO:0006457~protein folding	14	2.12E-05	2.36E-02

Table 3.5 Gene ID and annotation of 89 best predictor genes in OGS 3.2. Dmel_r5.42 are the up-to-date gene annotation in *Drosophila* genome (by May 2012). #N/A: not available in gene annotations or *Drosophila* orthologs.

Probe ID	GB Number OGS3.2	Gene Names	Dmel_r5.42_ Gene_ID	Dmel_r5.42 _Name
AM06501R	GB54890	kynurenine 3-monooxygenase	FBgn0000337	cn-PA
AM11801	GB46713	elongation factor 2	FBgn0000559	Ef2b-PA
AM12505	GB42011	follicle cell protein 3c-1	FBgn0000644	Fcp3C-PA
AM07018	GB48999	transcription factor ap-4	FBgn0001994	crp-PA
AM02905	GB44133	tubulin beta-1 chain	FBgn0003889	betaTub85D-PA
AM12856R	GB54085	#N/A	FBgn0004828	His3.3B-PA
AM04761	GB49031	ribosomal protein s18	FBgn0010411	RpS18-PB
AM08533	GB42312	mitochondrial isoform 1	FBgn0024891	ferrochelatase-PA
AM03767	GB40778	udp-n-acetylglucosamine transporter	FBgn0024994	Csat-PB
AM10675	GB52717	#N/A	FBgn0025709	CG8083-PA
AM10502	GB43265	equilibrative nucleoside transporter 1-like isoform 1	FBgn0026585	Ent2-PA
AM04357	GB43719	alpha-catulin-like isoform 3	FBgn0029105	alpha-catenin-related-PA
AM10585	GB43117	nucleoside diphosphate-linked moiety x motif mitochondrial-like	FBgn0030528	CG11095-PA
AM07719	GB44686	adp-ribosylation factor-like protein 16-like	FBgn0031254	CG13692-PA
AM05627	GB53009	gamma-aminobutyric acid type b receptor subunit 2	FBgn0031275	GABA-B-R3-PA
AM04152	GB52600	uncharacterized protein loc100871763	FBgn0031632	CG15628-PA
AM06376	GB54929	#N/A	FBgn0032005	Snx6-PB
AM05883	GB43205	uncharacterized protein loc100867964	FBgn0032598	ChLD3-PA
AM05890	GB43379	protease m50 membrane-bound transcription factor site 2 protease	FBgn0033656	S2P-PA
AM12313	GB41602	sugar transporter	FBgn0034045	CG8249-PA
AM02800	GB48112	abhydrolase domain-containing protein 16a-like	FBgn0035519	CG1309-PA
AM02667	GB46008	PREDICTED: adrenodoxin, mitochondrial-like [Apis mellifera]	FBgn0035529	CG1319-PB
AM07547	GB55515	inositol oxygenase-like	FBgn0036262	CG6910-PB

Table 3.5 (cont.)

AM10298	GB40580	integrator complex subunit 9	FBgn0036570	IntS9-PA
AM06329	GB49994	ribosomal protein l26e	FBgn0036825	RpL26-PA
AM10697	GB44936	histone-arginine methyltransferase carmer-like isoform 1	FBgn0037770	Art4-PA
AM09435	GB45806	unc93-like protein mfsd11-like	FBgn0038053	CG18549-PA
AM11809	GB50572	osiris 14	FBgn0040279	Osi14-PA
AM04616	GB42671	glypican 6	FBgn0041604	dlp-PB
AM02383	GB46479	uncharacterized protein loc100872175	FBgn0044324	Chro-PB
AM02866	GB42844	guanine nucleotide exchange factor dbs	FBgn0050440	CG30440-PA
AM08404	GB52980	probable g-protein coupled receptor 158	FBgn0051195	CG31195-PB
AM10876	GB47354	neurotrimin- partial	FBgn0051646	CG31646-PA
AM00120	GB51398	l-aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase	FBgn0052099	eap-PA
AM06335	GB44917	low density lipoprotein receptor-related protein	FBgn0052432	CG32432-PA
AM12190	GB51578	mitochondrial import inner membrane translocase subunit tim50-c-like	FBgn0250874	ttm50-PA
AM01698	GB52172	sh2 domain-containing adapter protein f	FBgn0259109	CG42251-PC
AM09976	GB40089	voltage-dependent l-type calcium channel subunit beta-2-like	FBgn0259822	Ca-beta-PI
AM02994	GB42487	calpain-c	FBgn0260450	CalpC-PA
AM05789	GB43054	#N/A	FBgn0261259	Fhos-PC
AM07970	GB47052	gene model 996	FBgn0261802	CG42748-PG
AM10360	GB40150	forkhead box protein p4	FBgn0262477	FoxP-PC
AM03831	GB53419	pyrazinamidase nicotinamidase-like	#N/A	#N/A
AM11223	GB45906	protein lethal essential for life-like	#N/A	#N/A
AM11755	GB55300	#N/A	#N/A	#N/A
AM06799	GB43761	uncharacterized protein loc100866401	#N/A	#N/A
AM08113	GB44455	poor imd response upon knock-in	#N/A	#N/A
AM02267	GB51435	#N/A	#N/A	#N/A
AM01635	#N/A	#N/A	#N/A	#N/A
AM09485	GB54184	bridging integrator 3 homolog	#N/A	#N/A
AM00574	#N/A	#N/A	#N/A	#N/A
AM01504	#N/A	#N/A	#N/A	#N/A
AM02307	#N/A	#N/A	#N/A	#N/A
AM09723	GB48133	l-lactate dehydrogenase a-like 6a-like	#N/A	#N/A
AM01423	#N/A	#N/A	#N/A	#N/A
AM00665	GB50486	#N/A	#N/A	#N/A
AM03859	GB41579	neuronal calcium sensor 2	#N/A	#N/A
AM02126	#N/A	#N/A	#N/A	#N/A
AM05033	GB49828	zinc finger cchc-type and rna-binding motif-containing protein 1-like	#N/A	#N/A
AM06506	GB50674	uncharacterized protein loc100879076	#N/A	#N/A
AM03212	GB43788	enhancer of split mgamma	#N/A	#N/A
AM07265	GB53750	upf0454 protein c12orf49 homolog	#N/A	#N/A
AM04631	GB40086	sodium-dependent multivitamin transporter	#N/A	#N/A
AM02964	GB50124	protein bv8	#N/A	#N/A
AM05101	GB52845	sh3 and cysteine-rich domain-containing protein 2	#N/A	#N/A
AM12118	GB41499	tetratricopeptide repeat protein 5	#N/A	#N/A

Table 3.5 (cont.)

AM11465	GB42877	polyamine-modulated factor 1	#N/A	#N/A
AM08986	GB44965	selenophosphate synthetase	#N/A	#N/A
AM02331	#N/A	#N/A	#N/A	#N/A
AM08728	GB45121	#N/A	#N/A	#N/A
AM00622	#N/A	#N/A	#N/A	#N/A
AM00499	#N/A	#N/A	#N/A	#N/A
AM12175	GB55541	ubiquitin-conjugating enzyme e2c-binding	#N/A	#N/A
AM12902R	#N/A	#N/A	#N/A	#N/A
AM01771	#N/A	#N/A	#N/A	#N/A
AM04023	GB46086	#N/A	#N/A	#N/A
AM08966	GB50806	#N/A	#N/A	#N/A
AM01678	#N/A	#N/A	#N/A	#N/A
AM02799	GB55582	#N/A	#N/A	#N/A
AM00664	#N/A	#N/A	#N/A	#N/A
AM00693	#N/A	#N/A	#N/A	#N/A
AM07754	GB52339	microtubule-associated proteins 1a 1b light chain 3a-like	#N/A	#N/A
AM07930	GB50946	uncharacterized protein loc100869298	#N/A	#N/A
AM10769	GB46912	tudor domain-containing protein 12	#N/A	#N/A
AM04830	GB50880	uncharacterized protein loc100866375	#N/A	#N/A
AM00646	#N/A	#N/A	#N/A	#N/A
AM08161	GB50625	#N/A	#N/A	#N/A
AM02489	#N/A	#N/A	#N/A	#N/A
AM03598	GB54137	transcription factor with ap2 domain	#N/A	#N/A

Table 3.6 Hormone signaling gene expression in (A) food scouting behavior, (B) nest scouting behavior. * Significant for "task" and "interaction", but not for "role", despite both significant in food and nest scouting. (C) Hormone signaling genes that were significant for effect of "role" between scouts and recruits (samples were pooled from both food and nest contexts, so here the expression patterns were an overall pattern across Table 3.6 A and B) ** Significant for the interaction between "role" and "context".

A.

Array probe	GB number (OGS 2.0)	Gene name	Expression pattern
08021	30298	Ecdysone receptor (EcR), transcription variation 1	Scout down
08226	17814	Hormone receptor-like 38, CG1864 * (probable nuclear hormone receptor hr38)	Scout down
09450	16873	Ftz transcription factor 1, CG4059, loc726450	Scout up

B.

Array probe	GB number (OGS 2.0)	Gene name	Expression pattern
09226	16648	Ultraspiracle Protein (USP), CG4380	Scout up
03985	11364	Ecdysone-induced protein 75, CG8127	Scout up
03986	11364	Ecdysone-induced protein 75, CG8127	Scout up
03987	11364	Ecdysone-induced protein 75, CG8127	Scout up
10132	16648	DopEcR, isoform A. loc413040	Scout up
03384	17814	Ecdysone-regulated gene E74	Scout up
08226	17814	Hormone receptor-like 38, CG1864 * (probable nuclear hormone receptor hr38)	Scout up
09450	16873	Ftz transcription factor 1, CG4059, loc726450	Scout up
06669	30150	Broad-complex (Br-c), CG11491	Scout up

C.

Array probe	GB number (OGS 2.0)	Gene name	Expression pattern
09226	16648	Ultraspiracle Protein (USP), CG4380 **	Scout up
03144	17330	Juvenile hormone acid methyltransferase, CG17330	Scout down
03985	11364	Ecdysone-induced protein 75, CG8127 **	Scout up
03986	11364	Ecdysone-induced protein 75, CG8127**	Scout up
10132	16648	DopEcR, isoform A. loc413040 **	Scout up
09450	16873	Ftz transcription factor 1, CG4059, loc726450	Scout up
06669	30150	Broad-complex (Br-c), CG11491 **	Scout up

APPENDIX A: LIST OF DIFFERENTIALLY EXPRESSED TRANSCRIPTS BETWEEN SCOUTS AND NON-SCOUTS

Probe ID	AM02601	AM08215	AM06839	AM01222	AM12535
AM02560	AM08731	AM02294	AM01496	AM04098	AM10392
AM08795	AM12568	AM03146	AM08389	AM10676	AM09821
AM00554	AM12055	AM04765	AM03042	AM09353	AM08421
AM08794	AM03683	AM11558	AM09330	AM06654	AM01581
AM05844	AM01970	AM12564	AM12813	AM01157	AM11737
AM09384	AM03570	AM08637	AM01899	AM07353	AM10755
AM12833R	AM12595	AM07466	AM12841	AM05169	AM02558
AM03144	AM02594	AM00447	AM09106	AM10132	AM10229
AM02123	AM08666	AM07141	AM03544	AM12292	AM11653
AM12832	AM07467	AM08764	AM00616	AM01461	AM08113
AM00637R	AM02752	AM02385	AM00762	AM09694	AM06727
AM03962	AM09116	AM10691	AM12862R	AM09280	AM01259
AM11377	AM08561	AM05149	AM11117	AM11290	AM09877
AM07878	AM02609	AM10732	AM03229	AM12348	AM05439
AM07292	AM05322	AM07688	AM06286	AM09136	AM07132
AM00684	AM03440	AM05221	AM02282	AM03579	AM07386
AM09467	AM11705	AM09388	AM03284	AM01661	AM07387
AM07033	AM02575	AM02131	AM07858	AM00569	AM08076
AM03110	AM09637	AM05271	AM10428	AM09961	AM06034
AM12877R	AM08958	AM08615	AM07064	AM08913	AM00766
AM12900R	AM05659	AM04049	AM01028	AM10539	AM01990
AM03715	AM01400	AM00886	AM11521	AM02942	AM11089
AM05029	AM01193	AM09159	AM12657	AM12045	AM11924
AM08218	AM12231	AM11890	AM04285	AM04003	AM04961
AM12598	AM01360	AM02706	AM07282	AM11795	AM06608
AM09062	AM09450	AM01611	AM05733	AM12338	AM06314
AM06567	AM05261	AM05154	AM02100	AM11301	AM12808
AM12778	AM12059	AM01692	AM09513	AM09599	AM05582
AM02905	AM02815	AM09605	AM09229	AM11557	AM03186
AM12832R	AM01267	AM02597	AM01709	AM09984	AM07901
AM12234	AM08593	AM01437	AM04268	AM05414	AM01839
AM12840R	AM10977	AM12783	AM04466	AM07838	AM08227
AM01901	AM12409	AM09282	AM07951	AM02567	AM03725
AM00906	AM00482R	AM12844	AM05259	AM03485	AM02387
AM01410	AM02224	AM01109	AM09135	AM12011	AM11230
AM05081	AM01750	AM08301	AM03116	AM09713	AM10278
AM05177	AM12884R	AM04716	AM12519	AM11616	AM07022
AM11925	AM11504	AM04602	AM02631	AM04451	AM09749
AM01522	AM10529	AM05192	AM08968	AM07769	AM02336
AM02077	AM00633	AM02232	AM11459	AM02021	AM03443
AM05315	AM01148	AM12836	AM07970	AM00536	AM02372
AM02667	AM09098	AM03209	AM02178	AM10510	AM01031
AM01720	AM05838	AM12900	AM12461	AM08232	AM11896
AM06498	AM12008	AM05853	AM01954	AM03545	AM00958R
AM07347	AM01965	AM07695	AM02904	AM05105	AM09767

AM07506	AM02219	AM02501	AM11646	AM01010	AM01566
AM01859	AM10759	AM09900	AM04826	AM12373	AM08856
AM03344	AM06927	AM09174	AM09148	AM06474	AM10272
AM01441	AM01005	AM03907	AM03972	AM08458	AM00518
AM12558	AM05900	AM05381	AM01568	AM00485	AM02507
AM07832	AM09740	AM07089	AM03455	AM11511	AM03285
AM12549	AM08254	AM06518	AM11477	AM03188	AM06810
AM06721	AM11319	AM01783	AM04235	AM02623	AM10285
AM08409	AM06318	AM08898	AM12745	AM05217	AM05950
AM03774	AM00705	AM02653	AM09529	AM11051	AM05410
AM12509	AM07693	AM01829	AM01651	AM05715	AM09963
AM05071	AM04610	AM10474	AM08709	AM03158	AM04593
AM00704	AM06148	AM09487	AM06783	AM00563	AM02818
AM01952	AM04973	AM03551	AM00640	AM06202	AM12844R
AM01523	AM07711	AM02259	AM11979	AM10255	AM01987
AM04064	AM08015	AM01911	AM04575	AM06902	AM07127
AM01929	AM02251	AM11525	AM12788	AM03846	AM06738
AM01572	AM01632	AM07889	AM12027	AM10280	AM05455
AM00717	AM01647	AM07320	AM10102	AM08992	AM10136
AM02725	AM00896R	AM02374	AM07407	AM01785	AM09064
AM07670	AM09341	AM02761	AM12470	AM08915	AM05793
AM04345	AM02017	AM03484	AM08741	AM10411	AM09358
AM10033	AM01382	AM10060	AM07067	AM11256	AM06526
AM05553	AM09206	AM03807	AM01968	AM09017	AM10537
AM06160	AM00002	AM02180	AM03850	AM03995	AM04655
AM08325	AM12882	AM12344	AM08245	AM07850	AM04323
AM05335	AM12866	AM00360	AM10059	AM01165	AM02914
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AM00635R	AM12303	AM12817	AM12703	AM03611	AM07552
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AM05123	AM08099	AM05839	AM09008	AM09770	AM10355
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AM12827	AM11165	AM03794	AM07949	AM02586	AM07864
AM10191	AM03211	AM11902	AM05393	AM02526	AM04538
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AM01318	AM12843R	AM01989	AM02754	AM07894	AM08083
AM08243	AM05531	AM05917	AM12515	AM09233	AM08100
AM10186	AM10138	AM03964	AM00733	AM04761	AM01416
AM08166	AM01795	AM12614	AM09538	AM01536	AM02153
AM06301	AM11585	AM05000	AM11030	AM08401	AM02218
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AM03749	AM04357	AM05446	AM00070	AM08628	AM01644
AM09072	AM02216	AM06296	AM01995	AM08362	AM12604
AM11721	AM08919	AM03048	AM05773	AM10123	AM02221
AM12058	AM09172	AM00452	AM02723	AM03784	AM02097
AM07696	AM01704	AM06375	AM08759	AM10464	AM03541
AM04850	AM09074	AM03591	AM10779	AM01313	AM11993
AM01904	AM09766	AM03405	AM05390	AM11684	AM02152
AM04497	AM01698	AM11364	AM11078	AM11370	AM05551
AM01327	AM07726	AM08266	AM10991	AM09761	AM09079
AM04766	AM00789	AM05584	AM01565	AM00352	AM10185
AM12850	AM12526	AM12385	AM02312	AM01747	AM10053
AM09738	AM11570	AM02167	AM02160	AM02417	AM02463
AM01617	AM01036	AM05718	AM09067	AM12118	AM08250
AM04629	AM09154	AM02134	AM10913	AM10969	AM11259
AM12368	AM01387	AM02356	AM04601	AM03029	AM06762
AM10238	AM06735	AM11119	AM11491	AM10267	AM05686
AM12829	AM09329	AM05144	AM02061	AM09790	AM12478
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AM12224	AM03353	AM11191	AM10242	AM11800	AM09857
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AM02301	AM08298	AM02129	AM02040	AM04192	AM00998
AM02358	AM01539	AM00855	AM03225	AM08124	AM04767
AM04303	AM01214	AM06024	AM03197	AM05063	AM07611
AM00362	AM04895	AM05654	AM06378	AM11261	AM02974
AM02722	AM01188	AM07880	AM11169	AM01241	AM08452
AM02279	AM02399	AM02195	AM02421	AM08139	AM06784
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AM06189	AM09163	AM01371	AM08697	AM09393	AM11279
AM04935	AM10738	AM01046	AM08918	AM03060	AM09852
AM11916	AM10116	AM00708	AM02284	AM02553	AM10771
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AM03270	AM07408	AM01235	AM05469	AM01414	AM12102
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AM02847	AM07344	AM05132	AM07918	AM12536	AM04139
AM00425	AM02920	AM01352	AM01685	AM03309	AM07412
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AM09052	AM04267	AM07547	AM06852	AM12513	AM11768
AM07940	AM01429	AM01149	AM02634	AM05808	AM02034
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AM01865	AM01312	AM01201	AM06887	AM02513	AM05884
AM11015	AM07812	AM07230	AM12699	AM08930	AM05184
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AM06187

APPENDIX B: NOVEL-FEEDER ASSAY EXPERIMENTAL SETUP

Figure B.1 Typical visual patterns used in the assay for training and novel feeders.

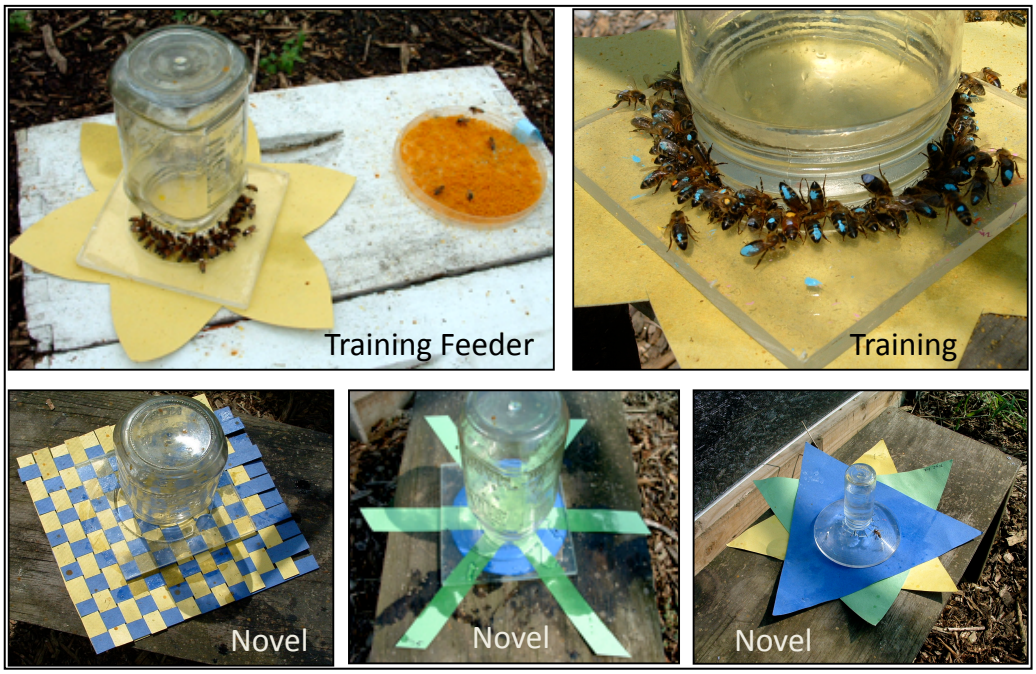

Figure B.2 Distinct patterns used as visual cues for novel feeders (2007 Experiment #1, colony R41). The dark stains were from sugar syrup spill during the experiments.

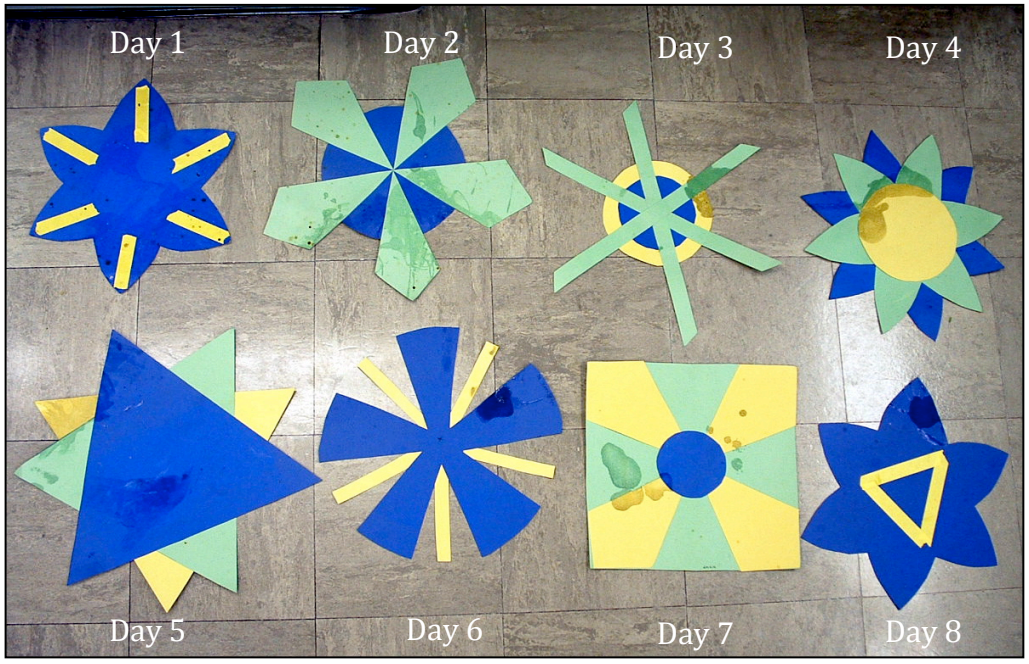

Table B.1 An example of the scents and paint colors used for the novel-feeder assay (2007 Experiment #1, colony R41). Those scents labeled with * did not seem to be attractive to bees, thus were no longer used after this experiment.

Day of Collection	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Site	NW corner	NE corner	SW corner	NE middle	SW middle	NE corner	NW corner	NE middle
Scent	Orange	Anise	Vanilla	Bergamot *	Cherry	Clove*	Almond	Cinnamon *
Paint	Red	Light Green	Orange	Purple	Pink	Copper	Grey	Rosy Orange